

Population planning gone awry

The United Nations should have known better than to arrange this month's conference on world population. For talking about population in isolation from development in general is a nonsense.

HERE is a cruel thing to say. The best hope for the World Conference on Population which began earlier this week in Mexico City is that it will merely be a waste of busy people's time. The danger is that, like its predecessor in Bucharest ten years ago, the effects of the conference will be positively harmful. The United Nations, which has organized the event, should in its own interests and for the sake of the good causes of international amity and of development which it espouses, resolve never again to inflict such an occasion on public attention.

The conference in Mexico City is not a scientific occasion, but one at which the organizers hope to translate knowledge and understanding into public policy, national and international. This recipe has worked well enough on some occasions, the Stockholm conference on environmental matters in 1972, for example. The conference on food, in Rome in 1974, was by contrast a platform for advertising the conflicts between developed and developing countries and a means of amplifying naive predictions that famine must lie ahead. The roots of this week's conference on population are similarly contaminated by unfounded opinions about the threat supposedly implicit in the rapid rate of population growth in many developing countries. Nobody can or will deny that there are many countries where serious social and economic problems are further aggravated by apparently uncontrolled fertility. Nor can it be disputed that there is a great and continuing need for a better understanding of the determinants of fertility. The issue is simply whether it can make sense to consider population policy in isolation from other public policies.

The difficulty is nicely illustrated by the material put out by the United Nations itself as background for Mexico City. The 1983 report of the UN Fund for Population Activities, for example, quotes from the World Population Plan of Action (adopted at Bucharest in 1974) the dictum that "the principal aim of social, economic and cultural development, of which population goals and policies are integral parts, is to improve levels of living and the quality of life of the people". But this will seem to most people a monumental tautology. How does the improvement of "levels of living and the quality of life of the people" differ from "social, economic and cultural" betterment, the presumed outcome of development? The implication that population "goals and policies" should be considered as integral parts of the process of development is nevertheless correct. The exercise at Mexico City, by singling out population policies for separate treatment, will do more harm than good.

Folly

The most obvious damage is that the United Nations will be thought to have put its weight behind the general but mistaken opinion that a rate of population growth that is anywhere greater than zero is an abomination, a proof either of fecklessness or of wilful irresponsibility. The truth is that the rate of population growth can be made an independent socio-economic variable only by artificial means, such as the edict of the People's Republic of China that families should have only one child or (worse) compulsory female sterilization. Where balanced attention is given to the "social, economic and cultural" aspects of development, the rate of population growth is, like the gross national product, a dependent variable, the product of the aggregation of decisions (or the lack thereof) about conception

made by individual couples. The experience of recent decades shows that government policies can indeed influence the ways in which couples behave, and that the consequences for the birth rate can be both rapid and beneficent. But the same experience also demonstrates the folly of attempting to force on reluctant governments, however hard-pressed, "population goals" whose wisdom is not self-evident.

There is now a wealth of data to illustrate how rapidly fertility rates can change. In the advanced economies of the world, fertility rates are near the level required for stability. Some of the governments affected profess anxiety that the proportion of the working population may be too small to support those already pensioned off, but few are seriously considering the restimulation of fertility — changing retirement policy seems safer — while migration is increasingly a safety valve. Similarly, among the Chinese and related populations of South-East Asia (but not in China itself), fertility rates have fallen dramatically to near-stability in a mere three decades, as also in Kerala, the Indian state also distinguished by its 100 per cent literacy rate.

So what can be done by the governments whose populations are growing at uncomfortable speed? The mere supply of contraceptives, or for facilities of abortion, will not check the growth of a population whose members do not appreciate the benefits that small families may bring. Especially when infant mortality is high (or has fallen only recently), it is asking too much of peasant families that their fertility should promptly be reduced to the net reproduction rate. Moreover, there is ample evidence that in the countries where population is least well controlled, in most of Africa for example, the chief cause of the agonizing social distress which abounds is not the rate of population growth as such but the indifference of governments to the importance of agriculture. India, well blessed though it may be with natural resources, is a shining example of how rapid population growth, recognized to be too high, may nevertheless partly be accommodated by giving agriculture the importance it deserves. What follows from all this is that population policies pursued in isolation are unworkable and even dangerous.

What can the governments of advanced societies do in such circumstances? The best course is to provide such technical and financial help as less fortunate governments may require, but otherwise to keep quiet. It is not nearly well enough understood that constant harping on the need to contain the growth of the world's population is understood by those to whom the message is addressed as patronizing neocolonialism of the most offensive kind. And appearance may be the reality. The projects supported by the Fund for Population Activities are both imaginative and welcome to the host governments, but the US Administration's intention, to be declared at Mexico City, that assistance will no longer be provided by the United States for projects involving abortion, might almost have been calculated to madden the governments of developing countries. Why should the manner in which the rate of population growth is controlled outside the United States be determined by the US Administration's felt need, in an election year, to follow an anti-abortion line? This divisive issue would not have arisen if the conference at Mexico City had never been arranged. The United Nations should learn from the bruising weeks ahead that it makes no sense to provoke trouble of this kind by offering a non-agenda for discussion. □



Japanese whaling

Will monitoring suffice when the hunting stops?

Tokyo

JAPAN'S Fisheries Agency has endorsed a new plan designed to keep Japan in the whaling business after the moratorium on whaling agreed by the International Whaling Commission (IWC) comes into effect at the end of 1985.

The plan, put forward by the "Study Council on the Whaling Problem", an advisory body to the chief of the Fisheries Agency, actually calls for Japan to end commercial whaling in accordance with the IWC ban. But it simultaneously calls for Japan to begin "investigative whaling" — the hunting of whales to "check scientifically on whale resources". And despite a statement from the Fisheries Agency chief that investigative whaling would "not be in search of profit but aimed at the assessment of resources", any real difference from present commercial operations has yet to be made clear. The same whaling fleet of one mother ship and four catchers would be used, and whales caught for "survey" would still be processed and sold for food. At the same time, Japan is to press the view that whaling operations within its own 200-mile zone should be allowed on the same grounds that the "aboriginal subsistence whaling" of the Eskimos of Alaska is allowed — that they are vital to the "social, economic and cultural life" of local communities.

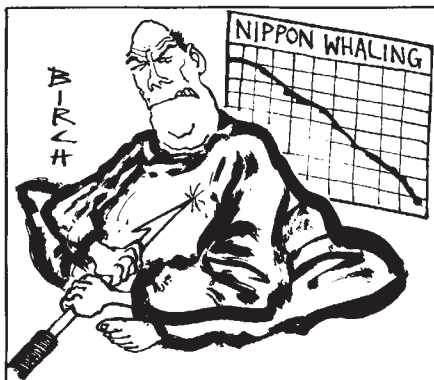
Behind all this semantic juggling lies the great sense of anger felt by the Japanese over what they see as the "moral imperialism" and "tyranny" of IWC — coupled with the knowledge that any direct defiance of IWC will result in the automatic imposition of economic sanctions against Japan by the United States.

The latest meeting of IWC, held in Buenos Aires in June, agreed massive cuts in whaling quotas for 1985, the last year before commercial whaling is due to be phased out. The quota for southern minke whales, which has accounted for 90 per cent of Japan's catch, is to go down from this year's figure of 6,655 to 4,224 — a level which will make it virtually impossible for the industry to continue operating at a profit next year, given that the quota must be shared with the Soviet Union.

In theory, Japan can simply lodge a formal objection to the quota and be freed from any obligation to observe it since IWC has no enforcement powers. Indeed, that is exactly what Japan did, along with the Soviet Union and Norway, when IWC first agreed the commercial ban in 1982. But the problem is that the Packwood-Magnuson amendment to the US Fisheries Act requires that any country defying IWC resolutions be penalized by cuts in their

fishing quotas in the US 200-mile zone. And those fish are worth far more to Japan than are the whales (120,000 million yen a year against 8,500 million yen a year). Japan's 1982 objection led to the imposition of a 10 per cent cut in the quota by the United States, and if Japan actually goes ahead and simply defies IWC then the fishing quota might be cut to zero by the United States.

The Japanese take particular offence at this year's IWC decision because they do not believe it was made on the basis of scientific evidence nor from a desire to keep whale stocks safe from extinction, but on moral grounds under pressure from conservationists. They point out that some members of IWC (membership is open to any country) have never had any financial interest in whaling and that Japanese surveys show there to be more than 400,000 minke whales in the Antarctic Ocean.



The Japanese, with their long Buddhist traditions, are not strangers to moral restrictions on the killing of animals. In the seventeenth century, one of Japan's more eccentric rulers, the fifth Tokuga Shogun, Tsunayshi, went so far as to ban the killing of virtually all living things and to require that dogs be spoken to in a polite manner — "honourable canine" being the preferred form of address. But having moral precepts forced upon them by another country is not something the Japanese can take easily. The hunting of whales has a long tradition behind it, and Japanese feel much the same way about the ban as an Englishman might if the supply of roast beef were to end as a result of economic pressure from the Hindu countries.

The official IWC view, however, is that its resolutions express scientific caution, not moral viewpoints. Nobody knows for certain how many whales there are and modelling population behaviour and the effects of whaling is fraught with difficulties: it is better to err on the side of caution than to drive a species to extinction.

What will happen next depends largely

on the United States, which has already indicated its willingness to hold a meeting of countries actually involved in whaling. If the United States prevails upon Japan to stop whaling, then it is likely that the Soviet Union will also obey the IWC moratorium for it relies upon Japan to buy virtually all the whale meat it takes. That will leave only Norway, which has a relatively small whaling operation. The Japanese are hoping that they can persuade the United States — whose fishing bans they can counter with bans on the import of US fish products — not to impose fishing sanctions and persuade IWC to agree to "investigative whaling" until the end of its moratorium in 1991. By then, Japanese whalers believe they will have enough evidence to persuade IWC that commercial whaling can be contained without risk to whale species.

Alun Anderson

Whitehead Institute moves in

THE Whitehead Institute for Biomedical Research began to occupy its handsome new building at the edge of the MIT campus in Cambridge, Massachusetts, last week with all the informality of a commune moving house. Graduate students and senior faculty alike were lugging boxes of equipment across the street. At one stage Dr Robert A. Weinberg, wearing a yellow and blue striped rugby jersey, was to be seen on the tailboard of a moving van protecting his rubber plant, now fourteen years old.

The controversies of three years ago, when the institute was founded with a benefaction of \$135 million by Mr Jack Whitehead, the founder of Technicon, sold to the Revlon cosmetics corporation in 1980, seem largely to have been stilled. Alarm that the Whitehead Institute might skew the pattern of biological research at MIT has been dissipated with the appointment of the first members of the institute, whose interests are as catholic as most people would wish. The agreement with MIT requires that faculty appointments should be jointly made, following strict MIT procedures, with seven years of probation for all but established people.

David Baltimore, the Whitehead director, says that he hopes to concentrate the institute's work in developmental biology, and that the number of faculty members should eventually double to 16. (Not unnaturally, he is now on the look-out for a drosophilologist with more than a nodding acquaintance with homoeotic genes.) Baltimore, still a little surprised that doors are repainted when he asks for that to be done, promises that he will spend as little time as possible in his large front office. One bone of contention with the biology department is that the Whitehead Institute is superbly equipped and enviably staffed with administrators. □

European Community

Davignon quits as commissioner*Brussels*

VISCOUNT Etienne Davignon, the Belgian who has masterminded the European Community's expanding science and research programme, will leave the European Commission at the end of the year. A pragmatist at heart, Davignon has let few opportunities slip to forge a solid base of international cooperation in research. It is not yet known who will be the new science commissioner.

The most recent example of Davignon's persistent efforts to mesh Europe's scientific talent to its industrial and economic needs is the £1,000 million Esprit information technology research programme.

Davignon has been forced to leave for two main reasons. First, the agreement of the ten member governments to support Mr Jacques Delors put an end to Davignon's hopes of becoming the new president of the Commission. Second, the Belgian Government made clear on 1 August that it would be putting forward the name of Finance Minister Willy De Clercq as Belgian member of the Commission. French-speaking Walloons claim that all Belgium's important foreign posts will be in the hands of Flemish speakers like De Clercq.

For the French-speaking south of the country, Davignon's portfolio, which includes the steel industry, is of vital concern. When Davignon joined the Commission in 1977, industry was his only responsibility. Since then, Davignon's workaholic drive gained him research and development sectors in both energy and science in the Commission of Gaston Thorn.

As part of his research responsibilities, he inherited the Commission's own Joint Research Centre (JRC) with some 2,200 personnel. Set up under the Euratom Treaty as a nuclear research centre, JRC had begun to diversify its activities after several years of crisis and indecision by the Council of Ministers. Crisis again struck in 1983, when the JRC Super-Sara project, a nuclear accident simulation experiment, was cancelled. According to Davignon, ministers had been shillyshallying for three years and were far too deeply involved in technical matters. The new JRC now boasts a board of governors and a scientific council to delegate decision-making.

Davignon applied the same philosophy — that ministers should be involved in policy, not in technical details — to the broader canvas of the Community's scientific research activity. His answer was the framework programme which sets out seven major goals for the Community's research, such as improving industrial efficiency, encouraging the quality of scientific collaboration and improving the environment. Research ministers would then have to assess and review resources to decide how programmes such as Esprit

would fit into this overall scheme of things.

The idea of the framework programme has been accepted in principle by the research ministers. But the necessary doubling of resources has fallen victim to the continuing squeeze on the Community's finances. Key elements of the framework programme — such as the European fusion programme, environmental research and the biomolecular programme — are already in place. Others, such as BRITE, designed to encourage

Romanian technology**Neglected by West and East**

THE United States Administration has been reconsidering the proposed sale of satellite monitoring equipment to Romania. The equipment, which would give the Romanians access to data from the "Landsat" satellite system, would constitute some of the most sophisticated equipment ever sold by the United States to an East European country. The sale was first proposed in 1977, but ran into opposition from some members of the administration who felt that the equipment could be adapted for military purposes.

According to US Government sources, the latest round of discussions has been little more than a routine review of the situation. In fact, the Romanian deal has taken on a new significance in view of the forthcoming sale of Landsat equipment to China. The technology package which it is expected the Chinese will purchase will give them access to data from both Landsat cameras, one capable of detecting objects some 100 m in diameter and one which will distinguish objects of about 30 m diameter. The proposed Romanian deal would include a ground station and computer bloc which would give access to the low-resolution camera only. There are fears that the Romanians might have the capability of converting such equipment to military use.

During the past two weeks there have been strong rumours of a disagreement between the Secretary of Commerce, Mr Malcolm Baldrige, and the Secretary of Defense, Mr Caspar Weinberger, over the export of the technology. Baldrige was one of the chief exponents of relaxing export control guidelines to allow the export of "dual-use technology" to China. Weinberger, however, is known to be uneasy and would like to have more control over the vetting of individual deals.

There is no doubt that the Romanians could derive considerable benefit from access to Landsat data. Although Romania participates in the Comecon *Interkosmos* programme, it is a far less active partner than, say, Czechoslovakia or East Germany, and there is no doubt that the

basic research and applications of new technologies to aid traditional industries, the biotechnology programme, telecommunications and the "stimulation" activity (aimed at raising the quality of multidisciplinary and multinational research), will follow in the coming months.

Where Davignon has made his mark has been in identifying Europe's weaknesses and energetically working at priorities to achieve closer scientific collaboration in the Community. If the quality of research is the key to industrial prosperity, the next generation will have reason to be thankful for his spell as commissioner.

David Price

Romanians were piqued when, after being given to understand that they were sixth in the running order, they were relegated to ninth place, either as a gesture of disapproval of President Nicolae Ceaucescu's independent foreign policy line or else due to an administrative decision that, after the first batch of cosmonauts (Czech, Polish, East German), the remaining six would go in (Cyrillic) alphabetical order.

Landsat data, with their applications to land-use and geological surveying, would be particularly useful for the Romanians, who, during the past decade, have seen their oil reserves dwindle to such an extent that the country is now a net energy importer. And with a failing agriculture, Romania has now embarked upon a "scientific nutrition" programme that will need every bit of technological help it can get. Even if the Romanian military did want to try to adapt Landsat technology for military use, they might well run into considerable opposition from the economic planners who have their own plans for it.

Vera Rich

Martin Goldman

MARTIN Goldman, one of the physical science editors of *Nature* in the 1970s, was killed in last week's train crash in Scotland.

Goldman joined *Nature* in 1975 after an outstanding academic career at Oxford, Princeton and Sussex, where he completed a DPhil in theoretical physics. He was attracted to science journalism by the opportunities that it provides for getting to grips with research on a broad front. He particularly enjoyed the opportunities that arose for writing popular pieces, and this experience, together with his occasional broadcasts for the British Broadcasting Corporation, led him eventually to the career in radio journalism on which he was well embarked. Based at BBC Radio Scotland, he had already established a national reputation as a science producer. He was 34, and leaves a widow and two young children.

David Davies

Cyclamates ban

Carcinogenicity claim questioned

Washington

THE safety of cyclamates, the artificial sweeteners, is again to be reassessed, this time by the US National Research Council. This turn of events has been prompted by a new study by the Cancer Assessment Committee of the Food and Drug Administration (FDA) which has found "very little credible data" to implicate cyclamates as carcinogens. The study report is also critical of the decision, in 1980, by former FDA Commissioner Dr Gere Goyan refusing an application to allow cyclamates back on the US market.

Since cyclamates were banned in 1969 on the suspicion that they might cause bladder cancer, FDA has been asked repeatedly by Abbott Laboratories Inc., the manufacturers, to lift the ban. In 1980, Goyan refused such an application on the grounds that studies with experimental animals had shown increased numbers of lung, liver, bladder and lymph tumours after the administration of cyclamates. Goyan also cited six cytogenetic studies showing increased numbers of chromosome aberrations.

FDA now questions not only the quality of the data cited in Goyan's judgement but also his interpretation of it. The cancer assessment committee complains that Goyan lumped together tumours at different sites so as to yield the appearance of statistical significance. The report also describes as "extremely misleading" his argument that if statistical analysis should suggest that the probability of a set of data representing the effects of an experimental treatment may have arisen by chance should be calculated as, say, 0.2, it would be fair to infer an 80 per cent probability that treatment and effects were causally related.

The committee also complains about Goyan's "uncritical" use of historical control data. FDA now says that data from different laboratories were used to control experimental data without allowing for differences between the spontaneous occurrence of tumours and differences in pathological procedures at the laboratories concerned. One study cited in Goyan's judgement is discounted because the results are "not a credible dose-response" and another, carried out at the Fondation Curie in Paris, because an on-site investigation found the quality control to be inadequate.

Goyan himself is unrepentant. He acknowledges that his 1980 decision was conservative but says he still feels that Abbott Laboratories had not discharged their burden of proof and that, if errors are to be made in matters of public health, they should be on the side of caution. Goyan says also that he is "peevish" that FDA had not informed him of the questions being asked about his judgement. Goyan stresses that the mutagenicity data, which have not

been re-examined, would themselves have been sufficient to prevent a relicensing of cyclamates.

Goyan's judgement is supported by Dr Marvin Legator, an FDA scientist during the Carter Administration. Legator says there are no new data to alter the position since then. He points out that new understanding of tumour initiation argues for rather than against the combination of incidences from different sites. The argument that studies should be discounted because they fail to show a credible dose-response curve is, he says, "bunk": many carcinogens produce plateaus or peaked curves in crude tests. Legator also argues that manufacturers have failed to provide basic data on metabolism of cyclamates, which varies markedly between individuals and at different times of day. It is not enough, he says, simply to fail to demonstrate unequivocally raised incidence due to cyclamate and its primary metabolite cyclohexylamine; the metabolic pathway must be understood in its entirety.

The National Research Council launched its arbitration between the new and the old FDA last week with an extraordinary public hearing, a cross between a scientific meeting, a public protest and an evangelical conversion drive. Most of the witnesses enthusiastically supported the new FDA review of Goyan's decision, but there was also some fear that the research council would be manipulated if it ruled only on carcinogenicity and failed to examine evidence for chromosomal damage and possible teratogenic effects. The chairman of the special panel appointed by the National Research Council Dr Richard Havel, said that his panel possessed the expertise to examine such data but gave no commitment to tackle the question explicitly.

One of Abbott's supporters in the drive to get cyclamates back on the market in the United States is the Calorie Control Council, which wants would-be dieters to have the choice of as many artificial sweeteners as possible: the argument here is that synergism between sweeteners means that by using combinations it is possible to reduce intakes of any single compound. The director of the council gave a rousing speech pleading for the return of this "very useful" compound.

Meanwhile, many FDA scientists feel that cyclamates, if they are carcinogenic, are certainly much less so than saccharin. All the available epidemiological evidence argues against a carcinogenic effect for cyclamates. On the other hand, many are convinced that there will eventually have to be a ban on saccharin. The newer artificial sweetener, aspartame, has a clean bill of health so far, but cannot be used in cooking or food products intended to be stored for long periods.

Tim Beardsley

Materials science

Academy backs synchrotron

Washington

WHEN presidential science adviser George Keyworth tried last year to secure funding for a National Center for Advanced Materials at Lawrence Berkeley Laboratory in California (see *Nature* 301, 456 and 302, 645; 1983), he was met with a hail of protests from materials scientists and Congress for having short-circuited the proper channels of peer review. Partly in response, Keyworth's Office of Science and Technology Policy commissioned the National Academy of Sciences to produce a certified, peer-reviewed list of priorities for major materials science facilities; that

Priorities for new facilities

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| 1. 6 GeV synchrotron | \$160 million |
| 2. Steady state neutron source | \$260 million |
| 3. 1-2 GeV synchrotron | \$ 70 million |
| 4. High intensity pulsed neutron source | \$330 million |

Priorities for upgrading existing facilities

1. Modifications to existing neutron sources at Brookhaven and National Bureau of Standards to exploit "cold" neutrons \$50-70 million
2. Incorporation where possible of insertion devices in existing synchrotron facilities (six facilities in all) \$20 million
3. Construction of an experiment hall and purchase of new instruments at the Los Alamos pulsed neutron source \$15 million
4. Improvements to the National Magnet Laboratory \$5 million
5. Development and installation of enriched targets for pulsed neutron sources, doubling the available neutron flux \$2-5 million

report* confirms what critics said at the time: there are more important things to do than build the synchrotron advanced light source that was to have been a major component of the Berkeley centre.

The good news for Keyworth is that now, armed with the academy report, he should face little opposition in pressing his materials science initiative — that is, so long as he modifies it to conform with the report's recommendations.

The 20-member academy committee that produced the report, after a reportedly gruelling effort to reach a consensus, gave its strongest endorsement to a new 6 GeV synchrotron radiation facility, estimated to cost \$160 million. This new generation synchrotron would, according to the committee, open up major new research

*Major Facilities for Materials Research and Related Disciplines, National Academy Press, Washington, DC, 1984.

areas by providing hard X-ray radiation at an intensity orders of magnitude greater than is now achievable. In particular, such a source could be used to detect elements in parts per million concentration and to identify their precise geometrical location in a molecule. One important application is in the discovery of geometric details of the active sites of catalysts and enzymes.

The 6 GeV synchrotron would be designed to take maximum advantage of the new "insertion devices" that can greatly boost the intensity of synchrotron radiation. Existing machines were designed and largely completed before the value of insertion devices was fully appreciated.

The committee's second-priority recommendation was for the construction of an advanced, steady-state neutron source that would provide perhaps ten times the intensity of present machines. Neutrons provide a unique probe of condensed matter by interacting weakly with atomic nuclei; neutron scattering has been used for example to determine the structure of magnetic superconductors and the spatial organization of macromolecules. US facilities are in the "dark ages" according to one committee member; by the mid-1990s, the earliest date

at which a new facility could be on-line, existing facilities in the United States will be nearly 30 years old.

The third-ranked priority is a synchrotron along the lines of the proposed advanced light source. This 1–2 GeV machine would be optimal for producing vacuum ultraviolet and soft X-ray radiation, of particular value in chemical physics and electron spectroscopy. Technological factors allow for radiation from such a machine to have a high coherence as well, favouring imaging applications such as microscopy and holography. (Although the administration has secured funding for the Berkeley centre — now known as the Center for Advanced Materials, without the prefix "National" — a decision on construction of the advanced light source was deferred.)

The committee also made a strong recommendation for upgrading existing facilities and for more money for small materials research programmes.

At least some of the committee's recommendations are expected to be incorporated into the fiscal year 1986 budget request now being formulated. It will be released by the President in January.

Stephen Budiansky

US manpower

Shortages of specialists foreseen

Washington

A SHORTAGE of aeronautical engineers and computer specialists in the United States by the year 1987 but large surpluses of life scientists are predicted in a report published this week by the National Science Foundation (NSF).

NSF is a respected name in the difficult field of manpower planning and its figures are likely to be seized upon by a variety of special interest groups during financial planning exercises this fall. The study explicitly addresses the manpower shortages likely to follow from the current planned increase in defence expenditure of 45 per cent in real terms over the period 1982–1987.

The period covered is from 1982 to 1987, and a variety of assumptions about economic growth and military spending are considered. The main results are little affected by the assumptions, however, and show clearly that a serious shortage of specialized engineers — thought by many to have been overcome in the past year or so — is still in prospect. The new analysis suggests that it will be possible to fill vacancies by retraining engineers and scientists from other disciplines, but that this will require serious additional expense and impede progress through productivity and quality losses. NSF expresses particular concern that a large switch in supply could lead to students forgoing advanced degrees to capitalize on job opportunities, with consequent difficulties for university recruitment.

While growth of jobs in engineering is expected to continue apace, led by electrical and electronic engineering, growth of employment opportunities in science is expected to slow down markedly from the 8.7 per cent annual average between 1977 and 1982 to only 3 to 4 per cent per year. Growth will be most rapid for computer systems analysts and social scientists, including psychologists. Agricultural and biological sciences show the smallest projected growths, with agriculture barely keeping pace with the growth of overall employment. On a simple supply/demand analysis the number of biologists by 1987 will exceed requirements by over 60 per cent, but the surplus is dramatically reduced when inter-professional mobility is taken into account. Mathematicians, in contrast, are likely to be much in demand, especially if defence expenditure increases as quickly as is planned. Technicians will also not have to look too hard for jobs, as their job opportunities follow closely those for engineers and computer systems analysts, where the defence built-up again will increase demand.

NSF declines to comment on policy options for containing the projected shortfall of electronics and aeronautical engineers. It prefers to remain a source of factual information and leave it to others to argue about action.

Tim Beardsley

**Projected response of the science, engineering and technical labor market to defense and nondefense needs: 1982–87. National Science Foundation.*

Computers

IBM in Europe

Brussels

TWO giant US companies, IBM and ITT, are to participate in the European Community's Esprit programme. The avowed aim of this strategic programme in information technology research is to help European companies to catch up with the US and Japanese penetration of the European home market and the world at large.

The announcement comes in the same week as the results of an investigation by the Commission of an alleged abuse by IBM of its dominant position in the European computer market.

An EEC spokesman was quick to dispel any idea of a trade-off between the two events. In the Esprit programme, IBM and ITT will participate through their European branches and will share with a number of other companies the results obtained.

More than 440 companies and universities will be collaborating in the first part of the main Esprit programme; an average of four companies (but sometimes as many as ten) will join in each project. About 200 million ECU (European Currency Units) or about £120 million has been put aside for the projects agreed to this year.

In the long-running IBM anti-cartel case, the Commission has reached a no-winners compromise with IBM. The Community wanted IBM to announce the technical details of new products as soon as they were advertised, to enable smaller companies to make compatible equipment. IBM has now agreed that such technical information will be released within four months of a new product being announced.

David Price

Bombs and rockets

THE offices of the European Space Agency (ESA) in Paris were open again on Monday after a bomb explosion at the weekend destroyed its public relations department, which was empty at the time. *Action Directe*, a French terrorist organization, claimed responsibility.

Some speculate that the fact that Telecom-1, one of two satellites launched by the rocket Ariane-3 from Kourou, French Guiana, on Saturday, would carry French military communications systems, had raised terrorist ire.

However, the launch of Telecom-1 and the European Communications Satellite, ECS-2, into geostationary transfer orbit went perfectly. On Monday morning, ECS-2's apogee motor was fired, injecting it into true geostationary orbit. The satellites appear to be functioning perfectly, but nobody at ESA was making firm predictions until the last circuit was switched on — just in case *Action Directe* had perfected a high-technology sting.

Robert Walgate

Soviet Union

More institutional inefficiency

THE current Soviet drive against inefficiency and corruption is throwing a disturbing light on the country's applied research structure. Since the early 1970s, party directives have urged close ties between research and production, and the establishment of "scientific-production unions" to serve the needs of the "scientific-technological revolution": it now appears that, far from speeding up the implementation of research in production, such "unions" can themselves be a cloak for slackness and corruption. Last month, *Pravda* drew attention to a particularly flagrant case — that of the "All-Union Scientific Research Institute for Potato-

based Food Products" — but in terms which made it clear that this exposé was meant as a warning to others to mend their ways. For many years, the usual explanation when production fails to match target has been that the scientists have done their part but there have been bureaucratic delays in implementing their recommendations. It was precisely to obviate these bottlenecks that the scientific-production unions were conceived. Yet the institute in question belongs to the union: indeed, its director, a certain A. Mazur, is also head of the scientific-production union. Nevertheless, and even though the institute is situated in Byelorussia, a renowned potato-growing region, the institute's record is appalling. In fifty years of existence, says *Pravda*, the institute has not published a single monograph, textbook or manual. The research projects supposedly taking place are overstaffed in comparison with similar projects abroad, where the results are ten times better.



On the other hand, the institute is said to be expert in paper shuffling and falsifying returns. After a number of commissions of inquiry had failed to penetrate further than the director's office, an authoritative investigation was initiated by the central committee of the Communist Party of Byelorussia.

It was found that most of the claimed "innovations" had never been carried through, and that the few which were implemented had an "economic effect" of

only a fraction of that claimed. Between them, over the past six years, the institute and the scientific-production union lost 1.1 million rubles by failing to install mechanized flow lines. Over the past two years, 1.6 million rubles were lost on projects which came to a halt at the "working out" stage. Conversely, one new installation was based on obsolete technology and was scrapped without ever going into operation.

At the same time, funds have been diverted and improperly disbursed, and the accounts falsified by as much as 667,000

rubles in a single year.

This particular institute has a key role in the much publicized "Food Programme" launched two years ago to bring Soviet nutrition up to Western standards by the end of the century. Swift action might therefore be expected to rectify such a deplorable situation. But, as *Pravda* points out, although six months have passed since the facts came to light, and although some of his subordinates have been demoted, Mazur is still in charge of both institute and scientific-production union. And, says *Pravda*, the scientific staff of the institute still have "no fresh thoughts nor original technical ideas" in their "scientific baggage".

Vera Rich

Japanese cities

Neoaquapolis

Tokyo

NOT to be outdone by ministries that see the future Japan populated by "teleopians" and "technopolitans" (see *Nature* 5 July, p.5), the Agency of Natural Resources and Energy has come up with a proposal for a "neoaquapolis" project in its annual "vision". Although it is not clear what is to be "neo" about the aquapolis, the project is based on more than the Japanese love of making up new words from foreign tongues (this one a mix of Latin and Greek). The proposal is that a marine city should be built on a man-made offshore island to help promote Japan's ocean development.

Marine development received tremendous impetus in Japan when the 1982 Law of the Sea Conference gave recognition to the establishment of 200-mile economic zones. With island territories scattered in a 2,800 km arc practically from Siberia to Taiwan — and the occasional convenient emergence of new islands as a result of undersea volcanic activity — Japan was found to rank second only to New Zealand in the increase in economic area this decision brought about. Japan's "economic" sea area is, at 4.5 million square kilometres, twelve times its land area and the sixth largest in the world.

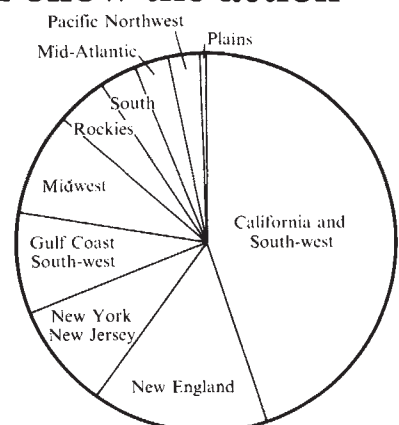
Research has been accelerated on a whole range of ocean resources — fisheries, including artificial fish pastures and the piping of light to the sea bottom to increase algal growth; seabed oil and minerals; wave power; deep-sea ocean thermal heat; the extraction of uranium from sea water; and survey and sensing equipment, including both satellite and buoy systems. National projects are also under way to develop manganese nodule mining techniques and sub-sea oil production systems which will sit on the ocean floor in deep waters. With government expenditure at around 60,000 million yen (£183 million) a year and large-scale investment by private industry, only the United States is putting more money into the oceans than Japan.

The sea is also being thought of as a place to store things. A project is under way to build huge synthetic skinned "sausages" which will float in the oceans full of oil. What more natural than to put some of the population in the sea too? Even building artificial islands turns out not to be a new idea — eleven have already been built and another five are on the way. The most spectacular, Port Island, off Kobe, has 20,000 inhabitants and is the world's largest container port. Costs have proved very high — around Y400 million an acre — and construction has only proved justifiable in areas that extend already very densely populated regions.

What the new report calls for is the vigorous promotion of industry-government cooperation to develop the advanced technologies necessary for a marine city to be built on an artificial offshore island. Exactly when the project might be feasible is not made clear. But in the meantime, the vision recommends that Japan team up with other countries to develop and build undersea robots.

Alun Anderson

Follow the action



INVESTMENT of venture capital in high-technology companies in 1982 in the various regions of the United States. Total investment increased from \$1,025 million in 1980 to \$1,822 million in 1982. Figures from *Technology, Innovation and Regional Development* published by Office of Technology Assessment, Washington, DC, July 1984. □

Whale-watching

Conservation goes commercial

Provincetown, Massachusetts

THE commercial exploitation of whales is once again a modest success in this small town, until a century or so ago one of the score of New England ports with a fleet of whalers on its books. But now the objective is not killing whales but watching them. At least four small enterprises operate diesel launches that offer visitors a chance to see a whale for between \$10 and \$13 a head. Business thrives, at least on clear days. The whales are not physically harmed, but what they think of the small flotilla scouring the nearby banks for a sight of them is not recorded.

The growth of this modest industry in the past eight years is not just a proof that conservation policy need not be an unmitigated drain on the public purse but also a neat demonstration of a symbiotic relationship between a commercial enterprise and an informal research enterprise — the Cetacean Research Program, based at Provincetown. The boat-owners give the whale-watchers access to their animals, not only off Cape Cod but, out of season, in the Caribbean. The conservationists repay this kindness by providing an intelligent running commentary on the proceedings.

Charles Mayo, the guide on one of the Dolphin Company's boats last week, and one of the founders of the Cetacean Research Program, has obviously learned in seven seasons to forestall the frustration of a hundred or so people who see nothing after four hours at sea. He emphasized from the start that on such a hazy morning, the chances of seeing a whale were bound to be small, especially because the upwelling of cold water off the submarine glacial moraines where the whales feed might turn the haze into fog. Obliging for the peace of mind of a hundred or so passengers, a fin-whale appeared right on cue, displaying its dorsal fin as if it were an oversized dolphin gambolling half a dozen times between five-minute dives.

Humpback whales (*Megaptera novaeangliae*), although on the average only 40 feet in these parts, are easier to see, at least on summer mornings, because of their habit of lying on the surface. They are also identifiable (to an enthusiast with a long telephoto lens on his camera) by the shapes of the white patches on their bellies and the flecks of white on their dorsal fins and tails. The Cetaceans have given them names — one of three lying half asleep on the surface was excitedly recognized as "Little Spot", a calf known to have been born early in 1979 in the waters around the Antilles. The other two, a mature female and a first season calf, declined to display enough of their tails for instant identification.

The Cetacean Research Program now reckons to have distinguished 230 humpback whales in the waters off the western edge of the Stellwagen Bank, many of

which have been also recognized at the southern extreme of their migration to the Caribbean (where they mate). Mayo is working on a detailed account of 213 of them for publication and says that the data he and his associates have gathered are the "best database on humpbacks in the world".

So are they still in danger of extinction? Mayo cannot be sure, but thinks the scanty evidence may be encouraging. The number of first-season calves recognized in the water increased from 4 in 1979 to 12 in 1983, while this year they have found a further 12 with three months yet to go before the annual migration south. Surprisingly, while calves return with their mothers in the second season, at two-plus (when suckling is over) two thirds have transferred allegiance to another female. As yet, there is nothing like a comprehensive survey of the humpback population off this north-east coast, while both fin and humpback whales from the Greenland populations may still be hunted legally.

The Cetacean Research Program is run on a shoestring, raising \$160,000 last year from the US branch of the World Wildlife Fund, a number of small foundations and by the sale of buttons on the boats for a tax-deductible \$2. Most of the money goes on film and equipment, with a tiny subsistence for those working far from home. The project would prefer not to have to ask the Dolphin Company for a commission for fear of compromising its independence.

There is also a petition to sign asking for a total ban on hunting and for the protection of critical whale habitats;

signatories line up in the inner cabin with studied nonchalance as if they were coming forward at a Billy Graham meeting. The hope is to better last year's roster of 45,000 signatures, which were "sent straight off to the [international] whaling commission".

This is the only hard-sell on behalf of conservation on the voyage, although it is occasionally let slip that this or that animal may have been killed before being able to return next year.

Much will no doubt depend on whether the whales regularly perform as well as they did last week. One fin whale first sighted straight ahead rushed by the Dolphin vessel at a distance of about 5 metres in an imitation of an animated nuclear submarine. Three minke whales (*Balaenoptera acutorostrata*) moved rapidly through the fog and long before half a dozen fin whales had been seen close to, some whale-watchers were hardened enough to keep their noses firmly in their books. Little Spot and her two companions were found on the return journey in almost exactly the same place, still half-asleep. One other humpback female some miles away, at first thought to have been Little Spot's mother (called Columbia), after imitating a 50-foot crocodile and a leaping dolphin, spread her tail above the water for long enough to have Mayo rushing about shouting "That's Ivory!" just as a man might recognize an old flame in the middle of nowhere. Ivory, he says, has been back with four calves since 1977, and now they hope she is pregnant again. If it goes on like this, the whales will be bumping into each other. Two of the Dolphin company's boats plying staggered shifts seemed to have some difficulty in avoiding a collision last week.

John Maddox

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 27 July	Change
14	6	Biogen (Switzerland)	8½	6¾	-1¾
2	1	Bio-Logicals (Canada)	1⅞	1⅞	-½
14 ⅜	7⅝	Bio-Response (USA)	8¾	8 ⅞	- ⅝
14	9¼	Cetus (USA)	11⅞	9⅞	-2
10⅜	4¼	Collaborative Research (USA)	5⅞	5¼	- ⅝
19⅞	11½	Damon (USA)	14¼	13	-1¼
26¼	11¾	Enzo-Biochem (USA)	15	12½	-2½
10 ⅞	4¼	Flow General (USA)	6½	4¾	-1¾
42¼	28¾	Genentech (USA)	34½	33	-1½
10¾	4½	Genetic Systems (USA)	5¼	5	-¾
17¼	8¼	Genex (USA)	9	11⅞	+2⅞
23	11	Hybritech (USA)	14	11¼	-3¼
16¼	7¾	Molecular Genetics (USA)	10¼	8¾	-1½
15½	8¼	Monoclonal Antibodies (USA)	12	8¼	-3¾
60⅞	39	Novo Industri A/S (Denmark)	44¾	40⅞	-4⅞
22¾	14½	Pharmacia (Sweden)	15⅞	17 ⅞	+1¼

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. Nature's weighted index of biotechnology stocks stood at 138 on 27 July, compared with 145 a month earlier. Data from E.F. Hutton, Inc.

East-West relations

SIR — Your editorial "How to order an East-West thaw" (28 June, p.735) shoots at the wrong targets. Its "sorry tale" of how East-West relations came to deteriorate to their present state is "familiar and depressing" only in its servile acceptance of every nuance of the official Reaganite version of events.

Having swallowed these myths it is perhaps not surprising that you are able to say that Washington is "about to try again for better scientific relations with the Soviet Union", as if throughout this administration nothing had been closer to President Reagan's heart (other than the odd anti-communist crusade) than better scientific relations. As the scientific community must be aware, nothing can be further from the truth.

It is the United States that has impeded scientific contact with the Soviet Union. It is the United States that has brought back cold war ploys to cut down the exchange of scientific information and created an atmosphere in which Soviet academics have been represented as red spies and some scientists have actually been mistreated by the US authorities.

The Soviet Union has always been against discrimination in the field of science and technology, and has more than 60 inter-governmental agreements with Western countries, covering the main areas and forms of cooperation in science and technology. As a result of these agreements, the countries concerned have 22 long-term programmes, some of them scheduled to last for decades.

The USSR Academy of Sciences has agreements on cooperation with scientific establishments in more than 100 countries. Some 500 Soviet scientists have been elected to membership of more than 200 national scientific organizations of other countries. Many of them are in the leadership of various international research centres. The USSR Academy of Sciences has over 80 foreign members from among the outstanding scientists of other countries.

This year marks 25 years since the signing of an agreement on the exchange of personnel for research purposes between the USSR Academy of Sciences and the National Academy of Sciences of the United States.

Despite the hindrances created by the last two US administrations, these contacts continue. Last year saw the end of a third Soviet-US Arctic expedition which collected important data on the study of whales and the fin-footed animals.

Encouraging results were received by the M.M. Shemyakin Institute of Bio-Organic Chemistry of the USSR Academy of Sciences, which cooperated with the Buffalo Medical Center in the United States, in the study of antibiotics and proteins effective against tumours.

In 1983 these establishments concluded a further five-year agreement on cooperation. Of world-wide interest is the exchange of research results between the Soviet Union and the United States in, for example, the fields of cosmonautics, thermonuclear energy and cardiovascular diseases.

Soviet science has achievements in many fields which it can share with other nations; it has more research personnel than any other country, about 1.5 million or 25 per cent of the world figure.

The mutual advantage of contacts in science and technology is endorsed by scientists and public figures of many Western countries, many of whom do not admire the Soviet Union's socio-political system.

Their opinion coincides with the Soviet Union's immutable stand on this question not because of political considerations, but because the growth of scientific and technological links promotes world progress, particularly international understanding and trust.

ALEXANDER SAPSAI
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● Mr Sapsai does not comment on one of the principal items raised by the article to which he refers — East-West misunderstanding (to say the least) over the treatment of Andrei Sakharov in the past two years — Editor, *Nature*.

Nuclear power

SIR — The British nuclear power programme was recently discussed extensively by Sir Alan Cottrell ("No Power of Persuasion", *Nature* 7 June, p.545). A review of an exhaustive three-volume survey of nuclear power technology edited by Walter Marshall, his article accords high praise to a work that is "an honest tale, plainly told", free of rhetoric, advocacy, passion or prejudice, in contrast to the propaganda world of the "Pied Piper" anti-nuclear lobby. The terms "military" and "weapons" appear nowhere in the review, neither in the description of contents, nor in the reviewer's own list of neglected topics.

Yet the military connections of the programme are indubitable. In the United States, the Calder Hall reactor is commonly known as a gas-cooled weapons reactor that made some electricity on the side. The continuing commitment of the United States to maintain its treaty rights to use British plutonium for nuclear weapons is a current issue.

J.R. RAVETZ

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AIDS priority

SIR — Some comments of Tim Beardsley ("Dispute over AIDS [acquired immune deficiency syndrome] patent priority" *Nature* 19 July, p.174) may leave your readers with the impression that human T-cell leukaemia virus III (HTLV-III) reagents were supplied to our group by Dr R.C. Gallo in our early investigations on lymphadenopathy virus (LAV). On the contrary, we supplied Dr Gallo with LAV first isolate on 17 July and 23 September 1983. According to Dr Gallo, the first sample failed to grow in his laboratory, but the second did.

HTLV-III was brought to our laboratory by a co-worker of Dr Gallo, Dr Sarngadharan, on 15 May 1984 for comparison with our own isolates. In our earlier investigations, we received from Dr Gallo only HTLV-I reagents, which were useful to show that LAV was not related to HTLV-I.

Our virus was deposited at the French National Collection of Cultures of Micro-organisms on 15 July 1983. It has since been freely available to any qualified laboratory and has indeed been sent to more than a dozen laboratories throughout the world.

Moreover, I disagree with the statement that "antibodies to core proteins are less well correlated with disease symptoms" than the p41 putative glycoprotein of HTLV-III.

Data published from our laboratory^{1,2} as well as from other laboratories³ indicate a high degree of correlation between antibody against the major core proteins of LAV and AIDS and pre-AIDS (lymphadenopathy syndrome). The lack of antibody to the p25 protein in the sera of some AIDS patients may be due to a profound impairment of their humoral response. Indeed we have observed a decrease or even a disappearance of antibody titre in some patients during evolution of the disease^{2,4}.

This phenomenon may be of prognostic value. Furthermore, the presence of antibodies to any of the LAV/HTLV-III antigens cannot be considered *per se* as a sign of AIDS or pre-AIDS, since a number of healthy homosexuals or haemophiliacs, as well as some asymptomatic carriers, have such antibodies. Although the incubation period of the disease may be very long, it is fair to assume that as in other viral diseases, the AIDS agent will not induce in all infected individuals a severe pathological disorder. The significance of antibodies to the various antigens of LAV/HTLV-III remains therefore to be determined, and is one of the real objectives of current research on AIDS.

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L. MONTAGNIER

1. Montagnier, L. *et al.* in *Human T-cell leukemia/Lymphoma viruses* (eds Gallo, R.C., Essex, M.E. & Gross, L.) 363-379 (Cold Spring Harbor Laboratory, New York, 1984).
2. Vilmer E. *et al.* *Lancet* i, 753-57 (1984).
3. Kalyanaraman V.S. *et al.* *Science* 225, 321-325 (1984).
4. Brun-Vezinet F. *et al.* *Lancet* ii, 1253-56 (1984).

Where cosmic rays come from?

The class of X-ray stars which are also sources of gamma rays, one the object called Geminga, shows how much there is to do before they can be called the source of cosmic rays.

If there were ever any doubt of the value in astronomy of ancient or crude observations of phenomena, it would surely be dispelled by the demonstration in this week's issue (p. 464) of how Bignami, Caraveo and Paul have been able to use a Chinese record dating from AD 437 to narrow down the choice of a model for Geminga, the γ -ray source. On the face of things, the tale resembles that of the identification of the Crab nebula as the remnant of a supernova observed by the Chinese a millennium ago, and recorded with sufficient precision for the identification to be convincing. The difference is that the star which surprised Chinese observers six centuries earlier is nowhere near a supernova remnant comparable with the Crab. That, so far as can be told, it lies close to the position now observed for Geminga proves nothing, but in the absence of other information and in the knowledge that Geminga must be an exceptional object, it is natural that people should put the two together.

Geminga itself requires more than casual explanation, as do the handful of other γ -ray sources now recognized. The obvious difficulty is that the γ -rays (whose spectrum extends above 50 MeV) are far more energetic than can be accounted for by thermal processes. With the rule of thumb that one electron-volt is roughly the equivalent of 10^4 K, γ -rays in this range could be generated only at temperatures of the order of 10^{12} K. And the mechanism invoked for the generation of X-rays in binary systems, involving the infall of material, usually from a companion star, onto the surface of a neutron star, with or without the annihilation of positrons and electrons near the surface, is similarly inadequate.

Although Geminga was recognized as a γ -ray source a decade ago, it is only now that enough data have accumulated to say much about it. The first discoveries of γ -rays were made from satellites (but have since been repeated at a higher energy by ground-based detectors). In the past five years, an object within the same half-degree region of the sky has been recognized also to be an X-ray source first by the Einstein satellite and, more recently, by Exosat. These are the data which now sustain the belief that Geminga is a periodic source of radiation with a period of almost exactly one minute. This was the development which led to the short burst of speculation, last year, that Geminga might be such an unusual object as to be respon-

sible for exciting the minute-long natural oscillation of the Earth by means of gravitational radiation.

Mistakes like that are by no means unexpected, given the difficulty of extracting data from objects such as γ -ray stars. The problems are well illustrated by the account of Bignami *et al.* with two associates, also in this issue (p. 481), of the variability with time of the X-ray emission from Geminga. Nearly ten hours of satellite observation appear to have sent only 700 useful X-ray photons through the Exosat detectors. Direct measurement of, say, the energy spectrum is only on the margin of what is possible. Wringing an estimate of the periodic fluctuation of the radiation from such an object is an act of faith. Yet Bignami *et al.* derive not merely the period but also the rate at which it has increased.

The extraction of a period from such data is necessarily a statistical exercise, and not a particularly objective one. What Bignami *et al.* have done is to fit the arrival times of the small bunch of photons they have been able to extract from the published X-ray data to various periods in the neighbourhood of 59 seconds. In doing this, the authors have plainly been looking over their shoulders at the chequered history of the supposition that Geminga is indeed a periodic source of X-rays first put forward in 1977 but withdrawn in 1981. Pending the accumulation of more data, it is natural that Bignami *et al.* should take on trust the previous estimate of the period.

In such circumstances, model builders plainly enjoy uncomfortably many degrees of freedom. Bignami *et al.* consider but reject the possibility that the Geminga source is an isolated neutron star. Even if such an object were able to convert energy into high-energy photons efficiently, it would have to be implausibly close to the Solar System to function as observed. If, on the other hand, the period of 59 seconds is not that of the primary rotation of a neutron star but the precession of its axis, there should be a second shorter period of fluctuation (not found in the data) and "conventional" pulsar radiation should be much more powerful — but even now, no radio pulsations from Geminga have yet been observed.

The authors settle instead for a model put forward two years ago to account for γ -ray emission from the X-ray source Cygnus X-3, involving a binary system of which one member is a neutron star. To fit the optical observations, its companion in

Geminga must be a dwarf star. But how can such a system have a period of mutual rotation less than a minute long? The explanation, according to Bignami *et al.*, is that the true period of orbital rotation is much longer, getting on for three hours, but that the neutron star component of the binary is the source of a beam of high-energy particles accelerated towards the more massive dwarf star and then converted by nuclear collisions into γ -rays and neutral pions. The period observed will thus be that corresponding to the beat frequency between the orbital and stellar rotation.

Precisely what should be made of this proposal is bound at this stage to be a matter of personal taste. With so few data, the remarkable feature of the situation is not that so much doubt persists but that anything worthwhile has been gleaned about Geminga. Moreover, there are now several testable proposals, of which the most intriguing may be the possibility that what seems to have been a once-and-for-all change in the rate of increase of the observed period may coincide with the surprising increase of the flux of cosmic γ -rays (with energies in excess of 10^{12} eV) early in 1980, well observed by ground-based detectors. A second coincidence would in the circumstances be almost clinching.

Of the puzzles that remain, the most puzzling is the origin of the object. Isolated neutron stars may be visualized easily enough as having begun their existence spinning much more quickly, and to have been progressively decelerated. If the period of fluctuation assigned to Geminga is indeed that corresponding to the beat frequency between two other rhythmic motions, it is a little easier to understand how the system may have been created comparatively recently. The possibility that the binary system was created in the collapse of an unstable massive star which somehow avoided a supernova explosion is attractive but, for the time being, untestable.

So why not wait until there are enough data to satisfy all reasonable curiosity? Because that will take many more X-ray satellites, and because the issue is potentially too important to be left in limbo. For there is a possibility that these objects are the principal source of cosmic rays in the galaxy. It would be a great convenience if that were the case.

John Maddox

Cancer

Functions of *ras* oncogenes

from Chris Marshall

ONE of the fundamental tenets of cancer research is that the conversion of normal cells to tumour cells is a multistage process. A possible mechanism would involve two or more genetic alterations, perhaps affecting different cellular functions. Studies with mesenchymal and epithelial cells in culture have provided some clues as to how this might work. They have identified two distinct differences between normal cells and tumour cells. The first is that tumour cells generally have the capacity to divide forever — they are immortalized. And second, their growth is controlled abnormally. Thus tumour cells, unlike normal cells, will often proliferate in low levels of serum mitogens or in the absence of anchorage. Frequently associated with abnormal growth control are changes in cell morphology. The existence of immortalized cell lines such as 3T3, which show normal growth control and are non-tumorigenic, and the demonstration for two tumour viruses, polyoma and adenovirus, that the functions of immortalization and abnormal growth control are genetically separate¹⁻³, suggest that different genes may be involved in conferring these two phenotypes on tumour cells.

The question then is, which genes in tumour cells are involved in immortalization and which confer morphological transformation and abnormal growth control? A year ago three papers published in *Nature* led to the same conclusion: activated *ras* oncogenes, detected by their ability to transform the established fibroblast cell line NIH 3T3, were not capable of immortalizing non-established cells but could morphologically transform and confer abnormal growth control on immortalized cells and, under certain conditions, non-immortalized cells⁴⁻⁶. A paper appearing in this issue of *Nature* comes to a different conclusion: under appropriate conditions, transfection with *ras* genes can lead to immortalization. These results clearly differ from those published previously, but they are still consistent with multistage models of neoplastic transformation because to produce the tumorigenic phenotype, both the expression and coding potential of the *ras* gene had to be altered.

Spandidos and Wilkie⁷ have transfected non-established strains of Chinese hamster and rat cells with mutant and normal *ras* genes either under the control of their own promoters or in plasmids that also contain transcriptional enhancers from simian virus 40 or the Moloney murine leukaemia virus long terminal repeat. They find that cells transfected with an activated c-Ha-*ras*-1 oncogene cloned from the T24 bladder carcinoma cell line are immortalized but not morphologically transformed or

anchorage-independent. When cells were transfected with the oncogene under the control of a transcriptional enhancer they were immortalized, morphologically transformed, anchorage-independent and tumorigenic. Further, while the normal c-Ha-*ras*-1 gene alone did not affect the cells, when coupled to a transcriptional enhancer it immortalized non-tumorigenic cells.

The reasons for the discrepancy between the earlier experiments and those of Spandidos and Wilkie are not entirely clear. Experiments using the same constructs but in Syrian hamster cells have not resulted in immortalization (R.F. Newbold, personal communication). The experiments of Spandidos and Wilkie, however, point to the importance of achieving the appropriate degree of expression of transfecting genes, and this degree may be subtly different in different cell types.

Another possible explanation for the different results is suggested by the observation that cells differ in their response to exogenously supplied transforming growth factors (TGFs) and that this reflects the ease with which they can be transformed⁸. Thus, differences in response to TGFs by different cell types may explain why, in some cases, a *ras* oncogene can morphologically transform non-established cells^{4,5}, while in Spandidos and Wilkie's experiments the T24 oncogene on its own did not. Interestingly, immortalization seems to be associated with an increase in sensitivity to TGFs⁸. Differences in responsiveness to TGFs and the degree of expression of a transfecting gene may also explain the intriguing observation that human fibroblasts are resistant to the effect of a transfecting *ras* oncogene⁹. Human fibroblasts are also resistant to transformation by Kirsten murine sarcoma virus (KiMSV)¹⁰.

What is not clear at this stage is whether experiments with *ras*-containing retroviruses really help to resolve the issue of whether *ras* immortalizes cells. Although inoculation of Harvey, Kirsten or BALB murine sarcoma viruses produces tumours, immortalization may not be a requirement for tumour formation by sarcoma virus because the virus stocks always contain a non-transforming helper virus, and a tumour can grow both by cell division and by recruitment from spreading viral infection. It would be of great interest to know whether the cells in these tumours have the ability to proliferate endlessly in culture. Infection of non-established rat embryo cells in culture with KiMSV only leads to morphological transformation at very low efficiency and such cells do not become immortalized (ref. 8 and B.W. Ozzanne, personal communication).

Nevertheless, it looks as though *ras*

genes, like the *myc* gene⁴, may function as immortalization genes. It is important to note, however, that none of the experiments reported so far has demonstrated that the immortalized phenotype of the transfected cells is directly under the control of the transfecting gene. This has been done so far only in the case of the large T gene of polyoma virus. Using a temperature-sensitive mutant it has now been possible to show that a functional large-T protein is necessary to maintain the immortalized phenotype¹¹. Such experiments are critical because of the propensity of rodent cells to undergo spontaneous neoplastic transformation. The rates of neoplastic transformation differ between species, for example, the frequency of establishment of cell lines from Chinese hamster is about 10 times higher than that of Syrian hamster cells¹².

Introduction of an oncogene into cells is only one way to analyse transformation. On page 508 of this issue, another approach is described. Stacey and Kung have micro-injected into various cell types purified p21 *ras* protein from BALB murine sarcoma virus expressed in *Escherichia coli*¹³. Unsurprisingly, though comfortably, micro-injection into NIH 3T3 cells results in morphological transformation. No transformation occurs when the protein is injected into non-established human diploid cells, a result which is reminiscent of those of Sager⁹ and Kopelovitch¹⁰. Of more novelty is the finding that when non-dividing NIH 3T3 cells are micro-injected with BALB MSV p21, the cells start to synthesize DNA. Such an effect is independent of serum growth factors since when quiescent NIH 3T3 or NRK cells are micro-injected in serum-free media with p21 *ras* derived from the T24 c-Ha-*ras* oncogenes, they undergo rapid proliferation (James J. Feramisco, personal communication). These results, coupled with the observations that *ras* expression varies through the cell cycle¹⁴ and that the p21 *ras* proteins interact with the receptor for epidermal growth factor¹⁵, suggest that one function of the normal *ras* gene product may be in transducing signals from growth factor receptors. Mutant *ras* genes may be capable of giving the signal in the presence of levels of growth factors which are insufficient to make normal cells divide. Whether immortalization depends on such a function of the p21 *ras* protein remains to be determined. □

1. Houweling, A. *et al.* *Virology* **105**, 537 (1980).
2. Rassoulzadegan, M. *et al.* *Nature* **300**, 713 (1982).
3. Treisman, R. *et al.* *Nature* **292**, 595 (1981).
4. Land, H. *et al.* *Nature* **304**, 596 (1983).
5. Newbold, R. & Overell, R. *Nature* **304**, 648 (1983).
6. Ruley, E. *Nature* **304**, 602 (1983).
7. Spandidos, D. & Wilkie, M. *Nature* **310**, 469 (1984).
8. Kaplan, P. & Ozzanne, B. *Cell* **33**, 931 (1983).
9. Sager, R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 7601 (1983).
10. Pfeffer, L. & Kopelovitch, L. *Cell* **10**, 313 (1977).
11. Rassoulzadegan, M. *et al.* *PNAS U.S.A.* **80**, 4354 (1983).
12. Kraemer, P. *et al.* *Cancer Res.* **43**, 4822 (1983).
13. Stacey, D. & Kung, H.F. *Nature* **310**, 508 (1984).
14. Campisi, J. *et al.* *Cell* **36**, 241 (1984).
15. Kamata, T. & Feramisco, J.R. *Nature* **310**, 147 (1984).

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Solar terrestrial physics

Global geospace study

from M.J. Rycroft, A.J. Smith, D. Jones, J.R. Dudeney and S.D. Shawhan

A MAJOR international programme to study 'geospace' is currently being planned by scientists in the USA, Japan and Europe. The term geospace was coined recently to describe the ensemble of separate regions close to the Earth and traditionally called the atmosphere, ionosphere, magnetosphere, magnetosheath and solar wind. The single word emphasizes that although these systems have until now been explored separately — for example, the magnetosphere in the International Magnetospheric Study (1976–79) and the stratosphere and mesosphere in the Middle Atmosphere Programme (1982–85) — they are in fact intimately coupled through a wide variety of mass, momentum and energy transfer processes and the time has now come to study them as a whole.

The Global Geospace Study (GGS) has two main scientific aims: to understand how energy from the Sun, particularly that carried by the solar wind and impinging on geospace, is transferred into, and converted by, the geospace system until it is eventually dissipated as heat in the ionosphere and upper atmosphere; and to understand the origin, transport and energy-gaining and -loss processes of the plasma populations of geospace. These questions are important for two basic reasons. First, they give insight into fundamental plasma physics. Some 99 per cent of matter in the Universe exists as plasma. The behaviour of cosmic plasma is not easily simulated in the laboratory, however, and geospace contains a vast natural plasma readily accessible both to detailed measurements *in situ* and to remote-sensing studies. The second reason is that an improved understanding of the response of geospace to solar variation has practical applications in radio communications, distribution of electrical power, geomagnetic surveys for minerals and hydrocarbons (particularly at high latitudes), space applications engineering and, possibly, the weather.

If the plans are approved, four spacecraft will be launched in the early 1990s to make concerted and comprehensive observations of four key components of geospace (see the figure). The spacecraft, known as Wind and Polar, will focus on the two sources of flowing plasma, in the solar wind and in the ionosphere and magnetosphere (particularly the polar regions), while the Equator and Geotail spacecraft will concentrate on the two principal energy-storage regions, the ring current and the geomagnetic tail. Extensive observations made from the ground using modern techniques and a strong theoretical and modelling effort complete the six components of GGS.

All the spacecraft will make plasma, par-

ticle and field measurements, though the exact specifications of their instruments (for example, energy range, sensitivity, frequency range) will be tailored to suit the particular region being studied. The spacecraft Wind, orbiting on the sunward side of geospace, will measure temporal changes of plasma density and velocity, ion composition, temperature and magnetic field strength in the solar wind input. The effects of these changes will then be measured on Geotail, Polar and Equator, and on the ground. For example, it is believed that a change in the interplanetary magnetic field embedded in the solar wind can trigger a 'substorm' in which energy stored in the geomagnetic tail is suddenly released into the auroral zones. This process might be observed by Geotail as a change in magnetic field direction and as plasma acceleration; the relative timing of these events will give information about the physical processes involved and their relation to one another. Increases in the energetic particle fluxes in the ring current and in plasma wave activity would be detected by Equator, enhanced auroral emissions in the polar regions by Polar, and ionospheric heating, electric currents, plasma flows and wave generation observed from the ground at high latitudes.

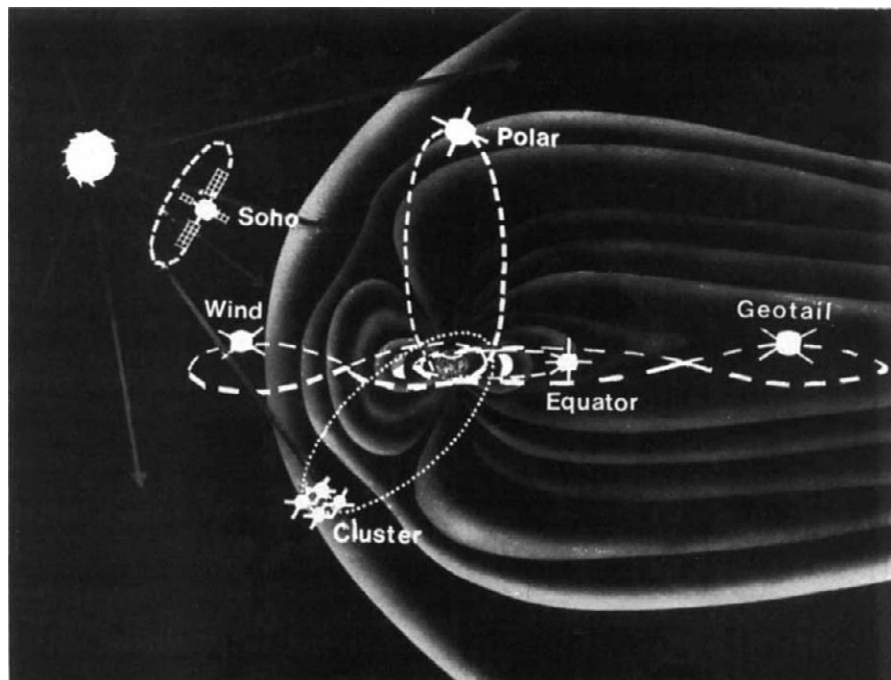
Three spacecraft (Wind, Equator and Polar) will be built by NASA and the fourth (Geotail) by the Institute of Space

and Aeronautical Science, Japan. The instruments will be provided by US and Japanese principal investigators working closely with European and American co-investigators. Four ground-based facilities have been proposed: the incoherent scatter radar at Sondrestromfjord, Greenland, a dual auroral radar network in the Arctic and a Canadian network of ground instruments for the Northern Hemisphere; and for the Southern Hemisphere, a comprehensive array of experiments at and around the British Antarctic Survey station, Halley (76°S, 27°W geographic).

In planning GGS, the importance of efficient and rapid handling, processing and dissemination of the data, using the most modern computer and communications technology available, has been recognized. Data from the spacecraft will be processed at a central data-handling facility before being sent to investigators, probably in the form of optical discs. Ground data will be inserted into the system in a similar way, the Antarctic data being transmitted from Antarctica via a geostationary satellite in order to be available rapidly.

GGS is a component of the even wider International Solar Terrestrial Programme, agreed by the space agencies of USA, Europe and Japan. Other space missions, which will concentrate on solar observations (Soho) and magnetospheric microphysics (Cluster) and will complement GGS, are currently being investigated by the European Space Agency. □

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Targets for spacecraft of the International Solar Terrestrial Programme. Wind will focus on the solar wind, Polar on polar regions of the ionosphere and magnetosphere, Cluster on magnetospheric microphysics, Soho on the Sun, and Equator and Geotail on the two main energy-storage regions, the ring current and geomagnetic tail respectively.

Stratigraphy

Carbon isotope anomalies?

from Michael A. Arthur

As stratigraphic resolution in some parts of the geological record improves, and sampling for stable isotope studies becomes ever more detailed, palaeoceanographers and geochemists are beginning to discover apparent evidence of major rapid changes in ocean-atmosphere chemistry. Many of these 'chemical events' are inferred from changes in the stable carbon isotopic composition of marine carbonate rocks and fossils, and the sulphur isotopic composition of marine evaporite sulphate minerals. In a stimulating paper, Magaritz *et al.* have suggested the discovery of the largest and most rapid enrichment of ^{13}C relative to ^{12}C (positive $\delta^{13}\text{C}$ shift) yet found in Phanerozoic marine rocks¹.

Their analyses of carbonate carbon isotopes were performed on samples from strata just below basal sequences through two Upper Permian evaporite deposits — the Castile Fm of the Delaware Basin, Texas and New Mexico, USA, and the Marl Slate of the Zechstein Series of north-west Europe. The data from both series, which are presumed to be equivalent in age, indicate the occurrence of a positive $\delta^{13}\text{C}$ shift of nearly 7 per mil, taking place in less than 4,400 yr in the case of the Delaware Basin deposits and 7,500 yr in the case of the Marl Slate. Relative timing of the rate of the shift was established using presumed annual varves in both finely laminated sequences. The authors argue that, for a variety of reasons, the $\delta^{13}\text{C}$ shift measured in these deposits accurately reflects an event that occurred in the world's oceans at that time. The implications of such an event for organic carbon burial rates and for changes in atmospheric ocean chemistry are staggering.

Shifts towards more positive (or heavier) $\delta^{13}\text{C}$ or $\delta^{34}\text{S}$ suggest, respectively, increased rates of burial of organic carbon and reduced sulphur whereas negative shifts suggest decreased rates of burial. Any such changes require net transfers of carbon (or sulphur) between the oxidized and reduced reservoirs of the element and transfer of oxygen (and CO_2) to or from the atmosphere. Therefore, these proxy records of geochemical transfers are important for consideration of short- and long-term changes in atmospheric chemistry, climate and the survival of life on Earth.

Many previous studies have focused on the apparent long-term stability of the carbon-sulphur cycles, and have proposed constancy in the mass of atmospheric oxygen on the basis of crossplots of average $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$, period by period (30 Myr or more). The crossplots of long-term averages of stable isotope data suggest that net transfers of carbon to the reduced

reservoir (organic carbon) are approximately balanced by net transfers of sulphur from the reduced (sulphide) to the oxidized (sulphate) reservoir, thereby maintaining oxygen levels in the atmosphere at some steady-state value²⁻⁴. Recently, however, higher-resolution time series of carbon isotope data have suggested that major short-term changes in the rate of organic carbon burial have taken place. For example, carbon isotope shifts of less than 1 per mil over a few 10^3 yr suggest increasing rates of burial of organic carbon of about twice the current rate during the glacial-Holocene transition^{5,6} and major increases in organic carbon burial rates over periods of a few 10^6 yr have been inferred from shifts of 1–3 per mil during parts of the Cretaceous and Cenozoic^{7,8}. These increased burial rates could have moderated the effects of proposed increases in CO_2 outgassing by volcanic activity^{9,10}. An important negative $\delta^{13}\text{C}$ shift of 1–2 per mil occurred over less than 10^5 yr at the Cretaceous-Tertiary boundary^{11,12}, perhaps signalling near cessation of productivity in ocean surface waters. A negative $\delta^{13}\text{C}$ of about 0.5 per mil near the end of the Miocene^{13,14} has also been documented in pelagic carbonate sequences.

The sudden increase in rate of organic carbon burial implied by the carbon isotope shift documented by Magaritz *et al.* is, however, difficult to accept and the cause difficult to envision, particularly because it is also suggested that the isotope event is not a short-lived excursion, but represents a sudden shift to a long-term high rate of organic carbon burial. Magaritz *et al.* also suggest that the lack of any sympathetic decrease in $\delta^{34}\text{S}$ values accompanying the $\delta^{13}\text{C}$ shift indicates the in-

dependence of the carbon and sulphur cycles on relatively short time scales.

The main question, though, is not so much whether the well-reasoned interpretations of Magaritz *et al.* are valid, but whether the samples they selected are appropriate for the type of analysis they wished to make. A critical problem in extracting primary geochemical signals from ancient sedimentary rocks is the ubiquitous spectre of diagenetic overprints. This problem may have haunted the efforts of Magaritz *et al.* to obtain high-resolution time series of stable carbon isotope data from varved sequences, particularly those containing significant amounts of organic matter. In fact, it appears that the shape of the $\delta^{13}\text{C}$ shift, which was detected both in the laminated, relatively organic-carbon-rich claystones and siltstones of the Bell Canyon Fm of the Delaware Basin and in the laminated organic-carbon-rich intervals of the basal limestone of the Castile Fm evaporites, may be an artefact of early carbonate diagenesis. Organic carbon values are as high as 5 wt per cent in the Bell Canyon Fm and as high as 9 wt per cent in the Castile Fm. The carbonate phase analysed by Magaritz *et al.* in these units consists primarily of euhedral rhombs of dolomite, which they state are characteristic of 'primary' precipitates; however, it is equally likely that the dolomite is an authigenic precipitate. Bacterial sulphate reduction and methanogenesis in the pore waters of the relatively organic-carbon-rich sediments may have caused important changes in the $\delta^{13}\text{C}$ of the total dissolved carbon, as well as changes in alkalinity which led to the precipitation of authigenic carbonate mineral phases with unusual carbon isotope values¹⁵.

The so-called 'smooth transition' from light $\delta^{13}\text{C}$ (nearly –3 per mil) in the Bell Canyon strata to heavy values (nearly +6 per mil) in the Castile evaporite carbonates may merely represent an increase in the importance of the primary evaporite carbonate with relatively heavy $\delta^{13}\text{C}$ values relative to that of isotopically-light authigenic phases. The carbonate content (as CaCO_3) in the Bell Canyon beds averages only 30 per cent, whereas that in the basal limestone is about 70 per cent. Moreover, the difference between $\delta^{13}\text{C}$ of organic matter and carbonate in the Bell Canyon Fm averages 27.4 per mil whereas that in the Castile is 30.5 per mil, again suggesting partial diagenetic alteration of carbonate in the Bell Canyon Fm. The evaporite carbonates of the Castile Fm may reflect something near the average carbon isotope value of later Permian surface seawater, but the carbonates of the Bell Canyon Fm are probably mainly authigenic.

Further support for this interpretation comes from unpublished stable isotope analyses of the uppermost shallow-water carbonates of the Permian Capitan Fm of the Delaware basin by R.K. Given and K.C. Lohmann. The uppermost beds of the

1. Magaritz, M. *et al.* *Earth planet. Sci. Lett.* **66**, 111 (1983).
2. Veizer, J., Holser, W.T. & Wilgus, C.K. *o eochim. cosmochim. Acta* **44**, 579 (1980).
3. Berner, R.A. & Rziswell, A. *Geochim. cosmochim. Acta* **47**, 855 (1983).
4. Mackenzie, F. & Pigott, J.D. *J. geol. Soc. Lond.* **138**, 183 (1981).
5. Broecker, W.S. *Geochim. cosmochim. Acta* **46**, 1689 (1982).
6. Shackleton, N.J. in *Fate of Fossil Fuel CO_2 in the Oceans* (eds Anderson, N.R. & Malahoff, A.) 401 (Plenum, New York, 1977).
7. Kroopnick, P., Margolis, S.V. & Wong, C.S. in *The Fate of Fossil Fuel CO_2 in the Oceans* (eds Anderson, N.R. & Malahoff, A.) 295 (Plenum, New York, 1977).
8. Scholte, P.A. & Arthur, M.A. *Bull. Am. Ass. petrol. Geol.* **64**, 67 (1980).
9. Berner, R.A., Lasaga, A.C. & Garrels, R.M. *Am. J. Sci.* **283**, 641 (1983).
10. Arthur, M.A. in *Climate in Earth History* (eds Berger, W.H. & Crowley, J.C.) 55 (Natl. Acad. Press., Washington, 1982).
11. Boersma, A. & Shackleton, N.J. *Init. Rep. DSDP* **62**, 695 (1981).
12. Perch-Nielsen, K., McKenzie, J. & He, Q. *Geol. Soc. Am. spec. Pap.* **190**, 353 (1982).
13. Vincent, E., Killingley, J.S. & Berger, W.H. *Mar. Micro.* **5**, 185 (1980).
14. Bender, M.L. & Keigwin, L.D. *Earth planet. Sci. Lett.* **45**, 383 (1979).
15. Anderson, T.F. & Arthur, M.A. in *Soc. Econ. Paleont. Mineral Spec., Short Course Notes* **10**, 1-1-151 (1983).

Capitan Fm are stratigraphically equivalent to the upper part of the Bell Canyon Fm. The $\delta^{13}\text{C}$ of the least altered Capitan Fm material, according to Given and Lohmann, is +5.2 per mil — very similar to the Castile Fm carbonate.

Therefore it seems that the rapid shift in $\delta^{13}\text{C}$ reported by Magaritz *et al.* is an artefact of diagenesis. The increase in $\delta^{13}\text{C}$

to the relatively high Late Permian values must have occurred earlier in the Permian or even over a longer period of time; thus, on the basis of data presently available, there is no need to postulate extreme fluctuations in rates of organic carbon burial over most of the Late Permian. □

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Gene transfection

A step nearer gene therapy?

from David Weatherall

THE last few years have seen spectacular advances in our understanding of the molecular pathology of some human gene disorders. It turns out that the commonest and most extensively studied of these conditions, the thalassaemias, in which there is defective synthesis of the polypeptide chains of haemoglobin, result from a series of diverse structural mutations of the globin genes^{1,2}. But while the work on the thalassaemias has told us a great deal about abnormal gene action and has led to the development of methods for prenatal diagnosis by direct DNA analysis, it has not resulted in any improvement in patient management. Not surprisingly therefore, thoughts have turned to the possibility of replacing the defective globin genes. Successful treatment of thalassaemias in this way represents a formidable task; nonetheless, a paper on page 476 of this issue of *Nature*³ makes a step in the right direction.

Before it will be possible to insert globin genes into blood cells with any reasonable expectation of correcting a thalassaemia mutation, a number of difficulties will have to be overcome. The recognizable red-cell precursors in the bone marrow are already terminally differentiated, that is they are programmed to go through several divisions and then to become mature red cells destined to be destroyed after about 120 days in the circulation. Hence the target for gene insertion has to be a haematopoietic stem cell, a self-sustaining population from which all the blood cells (including white blood cells and platelets) are derived. These cells constitute less than 0.1 per cent of the marrow cells, cannot be isolated as a pure population and can be assayed only in murine systems which are not applicable to human experimentation. Furthermore, even if it were possible to introduce genes into a proportion of stem cells there is no reason why these cells should proliferate preferentially; the defect in haematopoiesis caused by defective globin chain production is only apparent when the globin genes are activated in the terminally differentiated erythroid precursors. Moreover, there is no guarantee that the inserted genes would be expressed only in red cells and not in white cells or platelets. Even if they were

expressed in the appropriate cells their activity might not be synchronized with that of genes for other globin chains. For example, if β -globin genes were inserted to cure β -thalassaemia, it would be of little value if β -chains were produced more rapidly than their partner α -chains; the conversion of β -thalassaemia to α -thalassaemia would hardly be a *tour de force* for gene therapy.

Hitherto, these difficulties have been compounded by the fact that techniques for inserting foreign DNA into cells, using calcium microprecipitates for example, are extremely inefficient, achieving a transfection rate of approximately 1 cell in 10^5 . Thus, using the most optimistic calculations, we might need 10^9 marrow cells — about 10–20 ml of human marrow⁴ — to have a chance of inserting a new gene into 1 stem cell. It was against this unpromising background that a few years ago an attempt was made to insert a normal β -globin gene into the marrow cells of a patient with β -thalassaemia. Given the difficulties involved, it is not surprising that this premature experiment failed, although at least the effect of the bad press that followed was to clarify the criteria that would have to be met in any future attempts of this kind⁵.

The paper by Williams *et al.*³ in this issue of *Nature* suggests that at least one of the major obstacles to introducing foreign genes into marrow cells may have been overcome. Their elegant experiment entails inserting a marker gene into murine haematopoietic cells using a retrovirus vector and then following its fate using a well defined assay system for stem cells⁶. If mice are irradiated at an appropriate dose they die of marrow aplasia. However, if marrow is injected after irradiation the animals can be rescued. The donor marrow populates the spleen in the form of discrete haematopoietic colonies which have been shown to be the progeny of single cells. The latter, which thus have the properties of stem cells, are called colony forming units-spleen (CFU-S). The Boston group constructed a defective retrovirus vector containing a neomycin resistance gene (NEO) from Tn5 and a mouse cDNA sequence encoding dihydrofolate reductase. They then transfected mouse

marrow cells, injected them into irradiated mice and examined DNA prepared from spleen colonies by Southern blotting using a probe for the NEO sequence. About 10–25 per cent of the haematopoietic cells from the regenerating spleens contained the NEO-hybridizing fragment, suggesting that the recombinant genome had been transferred intact into a significant proportion of CFU-S. When CFU-S from these mice were transferred to a second group of irradiated mice, haematopoietic stem cells containing the fragment appeared in the spleens of the recipients, indicating that the transfected CFU-S had the capacity for self renewal. Preliminary analyses suggest a single site of integration per CFU-S.

These results are extremely encouraging. Together with the recent work of Joyner *et al.*⁷, who demonstrated the value of a similar but independently derived vector for gene transfer into mouse haematopoietic cells *in vitro*, they suggest that defective retrovirus vectors are able to achieve a very efficient level of integration into the DNA of haematopoietic stem cells, and that the transfected cells retain their capacity for self renewal. As the authors are careful to point out, many difficulties remain before this approach can be used for gene therapy. For example, nothing is known about the capacity for expression or regulation of genes inserted in this way, or about their structural integrity after multiple cell divisions. The safety and long-term stability of retrovirus-transformed cell populations will have to be explored. The present experiments were carried out in irradiated animals; it remains to be seen whether a system can be developed to promote preferential survival and proliferation of transfected stem cells in intact animals. Finally, up to 15 pg of globin may be needed per cell to correct a thalassaemia defect, so an inserted gene would have to be very efficiently expressed. Perhaps the most immediate application of the new approach will be for correcting enzyme deficiencies, where a much lower degree of expression of an inserted gene might be sufficient.

It is still early days, but the description of an efficient method for transfecting haematopoietic stem cells offers major encouragement to those who believe that the ultimate goal of clinical genetics is gene replacement therapy, rather than termination of pregnancy. □

1. Higgs, D.R. & Weatherall, D.J. in *Current Topics in Hematology* (eds Piomelli, S. & Yachnin, S.) 37 (Liss, New York, 1983).
2. Orkin, S.H. *et al. Prog. Hemat.* 13, 49 (1983).
3. Williams, D.A., Lenischka, I.R., Nathan, D.G. & Mulligan, R.C. *Nature* 310, 476 (1984).
4. Cline, M.J. *J. lab. clin. Med.* 99, 299 (1982).
5. Anderson, W.F. & Fletcher, J.C. *New Engl. J. Med.* 303, 1293 (1980).
6. Till, J.E. & McCulloch, E.A. *Ped. Res.* 14, 213 (1961).
7. Joyner, A. *et al. Nature* 305, 556 (1983).

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Island population biology

Possible effects of unrestricted pesticide use on tropical birds

from Jared M. Diamond

As the list of endangered species grows inexorably longer, announcements of yet another plant or animal being close to extinction have lost their shock value. It may still occasion surprise, however, when an entire fauna is found to be disappearing. Thus, J. Mark Jenkins' report (*The Native Forest Birds of Guam*, Am. Ornithologists' Union, Orn. Monogr. no. 31, Washington; 1983) on the decline of all native forest bird species on the island of Guam deserves mention — especially as it may portend similar catastrophes in other areas exposed to unrestricted use of insecticides.

Guam is a humid forested tropical Pacific Island of 209 square mile area, belonging to the Marianas group and administered as a US territory. It formerly harboured 13 native forest bird species, all but two of them endemic at the species or subspecies level to Guam or to the Marianas. Most of the species were common or abundant until the early 1960s. Their fates thereafter have been documented by roadside censuses carried out by government biologists, and by counts that Jenkins carried out in 1978 and 1979. In the late 1960s and early 1970s all native forest bird species went into decline, and four are now near extinction. The worst affected area is southern Guam, where nine species have disappeared completely and only one remains common. The surviving populations of most native species are now confined mainly or entirely to the northernmost one-tenth of the island, the rest of Guam having become virtually an ornithological desert for native species.

What happened? The usual explanations invoked to explain island bird extinctions — habitat destruction, introduced predators, storms and disease — do not work well for Guam. The island still has extensive areas of native forest, in which Japanese soldiers who refused to surrender in 1945 remained hidden until the last of them was captured nearly 30 years later. Forests sufficiently large and productive to support humans could surely support flycatchers and honey-eaters if there were no other problems. Furthermore, although habitats have been damaged by development, this has mainly affected the northern area where native birds are nevertheless doing best; with one exception the introduced predatory mammals, lizards and snakes have been present at least since 1890; and while Guam experiences periodic typhoons with windspeeds of up to 200 m.p.h., the native birds must have been exposed to such

typhoons throughout their evolutionary history. Introduced diseases are always a possible explanation, but there is no specific evidence for their role on Guam.

Several pieces of evidence implicate pesticides. The US military sprayed, dusted and fogged DDT weekly onto Guam (especially southern Guam) during and after World War II, and farmers in southern Guam carelessly applied large amounts of DDT in the 1960s. Bodies of the insectivorous swift *Collocalia vanikorensis bartschi* yielded DDE residues averaging 0.27 parts per 10⁹ (p.p.m.) in 1975, while guano collected

under swift nests yielded DDE residues increasing from 0 p.p.m. in the oldest layers to 0.1 p.p.m. in the most recent layers. Farmers, developers and the US military are still applying insecticides and herbicides today for pest and weed control. The insecticide hypothesis would explain why Guam's insectivorous birds have suffered steeper population crashes and more severe range contractions than have its omnivores and frugivores.

While many North Americans and Europeans feel that use of pesticides is subject to too few controls in their own countries, these agents are misused even more in some tropical countries without vocal environmental movements. If the insecticide theory for population crashes of Guam birds proves substantially correct, the recent fate of Guam's avifauna may find parallels elsewhere. □

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Semiconductor processing technology

New prospects offered by photolytic deposition

from S.J.C. Irvine

A Rank Prize Funds mini-symposium was recently held in Malvern on the topic of photolytic deposition*. Participants were brought up to date with the most recent advances in the use of lasers for a wide range of semiconductor device processing technologies, from laser writing of metal contacts less than 1 µm wide to laser-enhanced etching of gratings on GaAs.

A major innovation in the field has been metalization of semiconductors for microelectronics, such that metal films are accurately deposited from a volatile metal-organic source in a region of the substrate illuminated by a laser. This can be achieved by various mechanisms, including direct local heating of the semiconductor surface for pyrolytic decomposition of the metal-organic source and photolytic decomposition using UV lasers. R. M. Osgood (Columbia University) pointed out that while in photolytic processes, bond-breaking is more specific and sub-micrometre features can be written, pyrolytic deposition offers greater surface sensitivity and higher deposition rates. D. Bäuerle (Johannes Kepler University, Linz) uses an argon ion laser for writing nickel strips at scan rates in excess of 100 µm s⁻¹. High deposition rates allow fast scan rates and therefore short processing times for writing metalization structures. Deposition of the highest-resolution structures have been achieved, however, by photolytic decomposition of metal-organic adlayers.

D.J. Ehrlich (MIT Lincoln Laboratory)

*Photolytic deposition on metals, semiconductors and dielectrics, 24-27 April, organized by K. Ibbs and S.J.C. Irvine.

described how aluminium stripes, considerably narrower than the laser spot width, can be deposited by exploiting nonlinear effects in photodeposition. Aluminium lines are nucleated onto quartz using a UV laser and subsequently thickened by selective heating of the aluminium with a CO₂ laser. The thickening process occurs by the more rapid pyrolytic decomposition of triisobutyl aluminium. One interesting use of nonlinear processes is a photodesorption or photoactivation for patterning of a surface before deposition.

Two exciting developments reported at the symposium were the deposition of copper and platinum. Until now, it has been difficult to find suitable volatile precursors for photodeposition. Copper deposition was reported by S. Rolt of STL and by F.A. Houle of IBM, using acetylacetonate chelate compounds and illuminating the substrates with UV eximer lasers to photodissociate these precursors. Platinum photodeposition can be achieved through KrF eximer laser excitation of Pt(PF₃)₄ (I. Gianinoni, Max-Planck Institut für Quantenoptik, Garching). Clearly, future developments in the range of metals deposited by photolytic excitation will strengthen the applications of this technology.

Another new area of development, vital for the progress of photolytic processing, is the growth of epitaxial semiconductor films, where single crystal layers are photo-deposited onto lattice-matched substrates. The conditions for deposition are more

stringent than for polycrystalline growth and some surface heating is required to enable the surface atoms to order on the crystal lattice. Examples of these new developments are the growth of germanium onto sodium chloride substrates and of mercury telluride onto indium antimonide substrates. In a slightly different application of laser irradiation, H. Beneking (Institut für Halbleitertechnik, Aachen) described the epitaxial growth of gallium arsenide below 450 °C by metal-organic chemical vapour deposition combined with laser excitation of the growth surface to improve surface kinetics at these low temperatures.

The non-thermodynamic conditions created by photodissociation of precursors is attractive for deposition of insulators, such as SiO₂ and Al₂O₃, at high growth rates and at low temperatures. R. Solanki (Colorado State University) presented results on oxide growth over large areas showing good step coverage and low pin-hole density.

The breadth of semiconductor processing capability using laser photochemical techniques was an impressive feature of the symposium. The potential for maskless integrated device processing using a laser system is obvious, especially for device development or for custom devices. Lasers also have a place in more conventional processing, however; for example, high resolution etching of PMMA by photoablation using a 193 nm excimer laser pulse (G.M. Davis, University of Oxford).

Many more fundamental studies of surface photochemistry have been stimulated by the range of *in situ* analytical techniques available. R.P. Salathe (Generaldirektion PTT, Bern) reviewed *in situ* diagnostic techniques, such as electrical measurements, optical transmission and reflectivity, and luminescence measurements. Particular attention was paid to the problems encountered in studying films which are deposited over a small area (~1 µm) and the initial stages of growth. Studies of the morphology of growth can provide a valuable insight into laser/solid interactions with, for example, electric field enhancement created by certain sizes of surface features. Osgood described how holographic grating formation can lead to an electric field enhancement, for metal deposition, in the illuminated regions. This depends on the relationship between grating spacing and the wavelengths of the surface plasma waves.

Photolytic deposition of metals, semiconductors and dielectrics is a new field of research which is rapidly gaining momentum. Not only will it provide a means for exploring fascinating physical effects, but it will surely be an important processing technology of the future. □

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Molecular genetics

Ciliates come of age

from Joseph G. Gall

FROM what organism was a catalytic RNA molecule first isolated and characterized? Where can one find an animal that, at one time in its life cycle, has giant polytene chromosomes like those of *Drosophila* but at another time has each gene on a separate small molecule? In what organism does amplification of the genes for ribosomal RNA (rDNA) occur at a defined and inducible stage of the cell cycle? And finally, from what organism was a chromosomal telomere first isolated, sequenced and shown to function in a heterologous system? If you answered 'a ciliated protozoan' to each of these questions, you are probably one of the small band of biologists who already know the advantages of working with these unicellular organisms, and who attended the first International Conference on Ciliate Molecular Genetics*. For the benefit of anyone who was not there, this is what we talked about.

Nearly all aspects of ciliate research are dominated by the nuclear dualism of these protozoa — each unicellular organism contains one or a few diploid micronuclei (MIC) and usually one giant pseudopolyploid macronucleus (MAC). The MIC is largely inactive in RNA synthesis except for a brief period during meiosis; it divides in normal mitotic fashion at each cell division and is responsible for transmitting the chromosomes in a Mendelian fashion at the time of sexual fusion (conjugation). The MAC, on the other hand, controls essentially all 'vegetative' functions of the cell, most notably the synthesis of RNA. During cell division it pinches in half without undergoing a typical mitosis, and it breaks down completely during conjugation, when it is replaced by a new MAC derived from the zygote nucleus; thus, in normal conditions the MAC and MIC in the same cell start life with the same genetic constitution. For an introduction to these and other aspects of ciliate biology one should consult David Nanney's highly readable book, *Experimental Ciliatology* (J. Wiley, New York; 1980).

Beginning with the pioneering work of Nanney some 30 years ago, the focus of genetic studies in ciliates has shifted gradually from *Paramecium* to *Tetrahymena*. (At the Cold Spring Harbor meeting, Nanney, from the University of Illinois, discussed molecular data relevant to ciliate phylogeny, leaving the genetics to others.) It was clear from the presentations of Peter Bruns (Cornell University) and Ed Orias (University of California, Santa Barbara) that *Tetrahymena* is now quite

respectable from the standpoint of genetic maps, useful mutants and specially marked strains. Because the genetic constitution of the MIC is not critical to the cell, Bruns is able to map genes rapidly to each of the five chromosomes by putting MICs that are deficient in one of their five chromosomes into a cell with a complete MAC, using genetic tricks of his own design.

In contrast to the MIC, with its conventional genetic constitution, the MAC continues to bring surprises. It has been known for some time that the MAC of hypotrichs (*Oxytricha*, *Euplotes*, *Stylonychia*) contains separate gene-sized molecules, which emerge from an elaborate sequence of fragmentations and DNA eliminations, followed by the addition of C₄A₄/G₄T₄ repeats at the new telomeres. Much of what we know about the molecular biology of this process we owe to the group led by David Prescott (University of Colorado) and Prescott summarized the current information in his introductory lecture. L. Klobutcher (University of Connecticut) then reported that one MIC gene in *Oxytricha* contains three small inserts of 49, 49 and 32 base pairs (bp) within the coding region that are missing from the MAC copy and suggested that formation of the MAC involves throwing away some sequences within genes. The MAC of the holotrichs (*Tetrahymena*, *Paramecium*) undergoes less change than that of the hypotrichs, but studies from several laboratories, in particular those of M.-C. Yao (Washington University, St Louis), M. Gorovsky (University of Rochester) and E.H. Blackburn (University of California, Berkeley), show that MAC formation in *Tetrahymena* involves excision and ligation of sequences, as well as addition of telomeres with a characteristic and perfect C₄A₂/G₄T₂ repeat. Just where these repeats come from is not clear. Although there are internal clusters of C₄A₂/G₄T₂ in the MIC chromosomes, they are probably not the source of the new ends because neither are they perfect repeats, nor are they adjacent to sequences that are destined to end up near to ends. So perhaps, suggested Blackburn, the C₄A₂/G₄T₂ repeats are added sequentially by a non-template mechanism.

Ends of chromosomes, or more properly the ends of MAC DNA molecules, figured prominently in several other reports. D. Gottschling and T. Cech (University of Colorado) showed that about 100 bp (including the C₄A₄/G₄T₄ repeats) at the termini of the gene-sized molecules of *Oxytricha* MACs are resistant to digestion by micrococcal nuclease, suggesting that a special DNA-protein complex, distinct from nucleosomes and involving only 100 bp, defines the functional telomere in this

* The first International Conference on Ciliate Molecular Genetics at Cold Spring Harbor Laboratory, 2-6 May, was organized by Martin Gorovsky and Peter Bruns.

organism. J. Preer (Indiana University) and F. Caron (Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette) each reported that a gene coding for a surface antigen in *Paramecium*, the A antigen of *P. tetraurelia* and the G antigen of *P. primaurelia* respectively, was located near the end of a MAC chromosome. The situation is remarkably reminiscent of the variant surface antigens of trypanosomes, where the expressed copy of the antigen gene is similarly located next to a telomere. In *Paramecium*, environmental factors influence which of several non-allelic surface antigen genes is expressed, but there is no evidence that switching from one type to another involves transposition of the expressed gene copy, as it seems to in the trypanosomes.

Sequence data on the *Paramecium* surface antigen genes from both groups suggest some unusual codon usages; in particular, the translational stop codon TAA occurs in all three reading frames in putative exon regions. Direct sequencing of the mRNA by Caron confirmed the presence of UAA in the transcript. It has been known for some time that ciliate mRNAs are not efficiently used by heterologous *in vitro* translation systems, and the unusual occurrence of UAA may provide the explanation.

Two papers dealing with protein-DNA interaction were particularly worth noting. E. Niles (SUNY, Buffalo) identified a region upstream from the transcription initiation site in the *T. pyriformis* rDNA that contained ten tandem copies of a 16 bp sequence; the sequence is almost identical to the binding site for *Escherichia coli* catabolite activator protein (CAP) in the *lac* operon and CAP binds to the appropriate restriction fragments of the rDNA in the presence of cyclic AMP. These observations raise the interesting possibility that rDNA transcription in *Tetrahymena* is regulated by a CAP-like protein. O. Westergaard (Aarhus University, Denmark) looked for topoisomerase I activity in the rDNA chromatin of *I. thermophila*, using SDS-induced cleavage of the DNA as the assay. He found three regions in the central portion of the rDNA palindrome that were specifically cleaved, and each of these sites shared a common 16 bp sequence (different from that observed by Niles). The function of the presumed topoisomerase is unknown. The cleavage regions are upstream from the site of transcription initiation, but they are also not far from the origin of replication.

Tetrahymena offers unique and nearly untapped opportunities for studying the molecular events of meiosis, since along with certain fungi and algae, it is one of the few organisms from which one can obtain large amounts of precisely staged meiotic material. D. Martindale (McGill University) used this feature to prepare cDNA clones of genes that are expressed only during meiosis. One of these, a non-repetitive gene present in both MICs and MACs,

cross-reacts with sequences in yeast and *Drosophila*. A cross-reacting genomic clone from yeast hybridized to several yeast RNAs, one of which was strongly induced during sporulation. A cross-reacting *Drosophila* clone was localized to the region of *zeste* by *in situ* hybridization to salivary gland chromosomes; restriction enzyme mapping showed that the *zeste* gene itself is probably involved. *Zeste* has been implicated for some time in somatic chromosome pairing in *Drosophila*. These new results from *Tetrahymena* suggest that meiotic and mitotic pairing in widely diverse organisms may involve homologous proteins.

As in other ciliates, conjugation in the hypotrich *Euplotes* is induced when cultures of different mating types are mixed. Unlike *Tetrahymena* and *Paramecium*, however, *Euplotes* forms both homotypic and heterotypic pairs. P. Luporini and C. Miceli (University of Camerino, Italy) have purified heat-stable glycoproteins, called gamones, which induce homotypic pairing

when added to a culture of different mating type from that used as the source of the gamone. Gamones are active at concentrations in the picogramme per ml range, consistent with the needs of such free-living organisms in nature. Following the original suggestion of A. Miyake (University of Camerino), gamones are believed to be recognized by receptors on the surface of the organism, a primitive system for discrimination between 'self' and 'non-self'.

Many diverse topics were touched upon in this useful symposium. Only a few papers were directed primarily to ciliate specialists; an impressive number illuminated questions of general importance for eukaryotic molecular biology. The study of ciliates is rapidly coming of age, and we can expect an increasing number of workers to use these fascinating protozoa as experimental material in the near future. □

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100 years ago

SEATS IN RAILWAY CARRIAGES

In a recent article in *Science et Nature* the writer, after animadverting on the lateness of the day at which shoemakers have at length begun, though still very imperfectly, to take account of the osseous framework of the human foot, proceeds to investigate the relation between the structure of the human trunk and that of the seat, more particularly in railway carriages, designed for its accommodation. In a sitting posture the pelvis has for its sole function the support of the upper part of the body. The spinal columns, however, is inserted in the pelvis, not in the form of a straight line but of a curve (Fig. 1). This inflection on the part of the backbone, while adding to the mobility of the trunk, imposes on it the necessity of a continual balancing movement, the centre of gravity being shifted every time the head and thorax sway to one side or the other. Such balancing movement is necessarily also attended by a certain expenditure of energy. To allow the upper part of the body to remain comfortably at rest there must be supports for the back, the shoulders,

and the head. So far as these are wanting, the body will tend of itself, unless counteracted by an effort of will and nervous force, to bend forward, till at last the forehead finds the knees to lean on. The position of the body in sitting is all the easier, and its rest all the more complete, the more decided is the inclination of the back of the seat and the more obtuse is the angle formed by the trunk and the thighs. Seats such as the *dormueuses* realise the most favourable conditions in this respect.

Fig. 2 represents a man comfortably seated and propped. The back of the seat supports him principally under the shoulder-blades, offers the chest a depression to sink in, and altogether keeps the upper part of the body in a free and easy position. Fig. 3 shows the same person in a

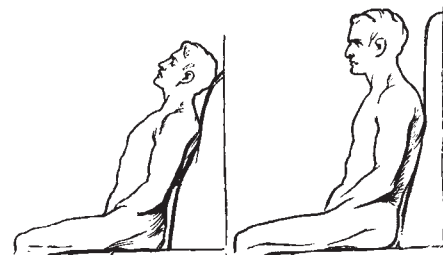


FIG. 3.

FIG. 4.

similar position, but with his head resting behind. In both these figures the back of the seat is seen exactly, in profile, and to the writer of the article such seems the construction which is most convenient in railway carriages.

Fig. 4, on the other hand, represents the profile of a man seated as passengers are in many of our actual first-class carriages. His position is perceived to be a forced one in contrast with that just noticed, and altogether disagreeable.

Under the actual conditions, such as they have been described, what becomes of the traveller when sleep at length overtakes him? Little by little he slides down on his seat till the lower extremity of his shoulder blades, which has most need of support, finds the most sensible projection. Lastly, the head inclines forward or to the side, if it does not bury itself in the breast.

From *Nature* 30, 319, 31 July 1884.

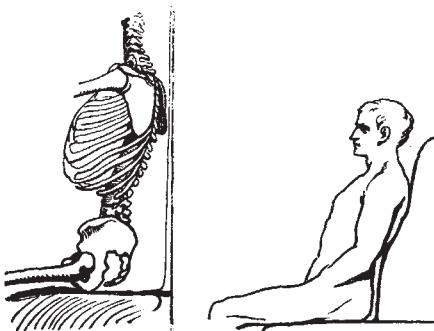


FIG. 1.

FIG. 2.

Nuclear war — counting the cost

SIR — Recent papers on the consequences of nuclear war^{1,2} have not attempted to relate their findings to historical events. There are at least three events that appear relevant. The ozone depletion from the 1908 Tunguska meteor was found by Turco *et al.*³ to be comparable with that expected from a 5,000–10,000 megaton nuclear war. Stothers' study⁴ of the volcanic dust cloud of AD536 found its optical depth to have been between 2.2 and 2.5 and its duration to have been approximately one year, comparable with nuclear war models. The intense incendiary attacks on Japanese cities during the last months of the Second World War offer a test of the injection and retention of large quantities of urban smoke in the atmosphere. All three of these events could conceivably be used to normalize the models to actual events but the smoke study requires the use of recent meteorological and astronomical data.

The measured area of the total destruction by fire from the well documented USAAF attacks on Japan between 9 March and 18 August 1945 was 178 square miles (464 km²)⁵ or 1.8 per cent of the burned area of the smallest attack (100 megaton cities only) necessary to produce a nuclear winter¹.

Eddy *et al.*⁶ noted that 0.1 to 0.2 per cent changes in solar output, as measured by spacecraft, can be related to the fraction of the solar disk covered by sunspots. By using spectral analysis techniques appropriate to low signal to noise ratio signals, Currie⁷ discovered that between 1870 and 1977 there existed an inland temperature variation of 0.18°C with a frequency matching that of the solar cycle. Eddy demonstrated⁶ that such a variation can be driven by sunspot blockage of the solar disk. Since the nuclear winter implies a near total blockage of sunlight, the much smaller attacks on Japanese cities can be expected to produce an effect comparable to the changing sunspot areas but to my knowledge there have been no attempts to investigate the historical record for such an effect.

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1. Turco, R.P., Toon, O.B., Ackerman, T., Pollack, J.B. & Sagan, C. *Science* **222**, 1283–1300 (1983).
2. Covey, C., Schneider, S.H. & Thompson, S.L. *Nature* **308**, 21–25 (1984).
3. Turco, R.P. *et al.* *Science* **214**, 19–23 (1981).
4. Stothers, R.B. *Nature* **306**, 344–345 (1984).
5. Mac Isaac, D. *The United States Strategic Bombing Survey*, Vol X, (1976).
6. Eddy, J.A., Gilliland, R.L. & Hoyt, D.V. *Nature* **300**, 689–693 (1982).
7. Currie, R.G. *J. Atmos. Sci.* **38**, 808–818 (1981).

SIR — The known behaviour of smoke in the aftermath of large fires raises questions about the climatic conclusions of Turco *et al.*¹ and Covey *et al.*². The recent work of Stothers³ suggests that a 90 per cent reduction of solar insolation (even over several months) will not cause freezing surface

temperatures. Even regionally persistent smoke (one month duration — from near-by forest fires) does not cause surface freezing⁴, contradicting the claim of Covey *et al.* that smoke clouds persisting over a region for a few days would do so.

Covey *et al.* also note that a major uncertainty in "nuclear winter" is the extent of prompt smoke removal. This effect is demonstrated by the "black rain" following some firestorms during the Second World War^{5–7}. Large fires themselves can cause thunderstorms⁸.

A major factor in the atmospheric residence time of smoke in the atmosphere is the altitude to which it rises. Turco *et al.* allow for firestorm smoke to enter the stratosphere, but none of the Second World War firestorms lofted smoke to such heights^{5–9}, nor did much larger (approximately 1,000 km²) natural firestorms¹⁰.

The great spread of forest fire smoke (several thousand km distance) discussed by Crutzen and Birks¹¹ may not be typical. Special upper-atmospheric circulation patterns are credited with both the great distance covered by the 1918 Minnesota fire smoke pall¹² and the transoceanic spread of smoke from the 1950 Alberta fires^{13,14}. Smoke from nuclear fires may not easily spread far beyond its origins.

Turco *et al.* consider 100 million tons of smoke sufficient for major hemispheric-scale climatic perturbations. Such a total (or significant fraction thereof) could have been reached during historic fires, evidently without climatic perturbation. Areas of over 25,000 km² have burned simultaneously¹⁵. Masses of 4.2 g cm⁻² are possible for mature forests^{16,17}. Assuming 3 per cent smoke emission and 2/3 fuel consumption (possible for high-intensity fires¹⁹), more than 20 million tons of smoke particles can be generated in only one such natural fire.

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1. Turco, R.P., Toon, O.B., Ackerman, T., Pollack, J.B. & Sagan, C. *Science* **222**, 1283–1300 (1983).
2. Covey, C., Schneider, S.H. & Thompson, S.L. *Nature* **308**, 21–25 (1984).
3. Stothers, R.B. *Nature* **307**, 344–5 (1984).
4. Boerker, R.H.D. *Behold Our Green Mansions*, 149 (University of North Carolina Press, 1945).
5. Caidin, M. *The Night Hamburg Died*, 94 (Ballantine, New York, 1960).
6. *U.S. Strategic Bombing Survey* Vol. X, 96–97 (1976).
7. *Hiroshima and Nagasaki*, 88–94 (Basic Books, New York 1981).
8. Vogl, R.J. in *Fire and Ecosystems* (eds Kozlowski, T.T. & Ahlgren, C.E.) 153 (Academic, New York 1974).
9. Irving, D. *The Destruction of Dresden*, 175 (Ballantine, New York, 1963).
10. Pyne, S.J. *Fire in America*, 300 (Princeton University Press, 1982).
11. Crutzen, P.J. & Birks, J.W. *Ambio* **11**, 115–125 (1982).
12. Lyman, H. *Mon. Weath. Rev.*, 509 (1918).
13. Bull, G.A. *Met Mag.* **80**, 3 (1951).
14. Smith, M. *Mon. Weath. Rev.*, 193 (1950).
15. Pyne, S.J. *Fire in America*, 506 (Princeton University Press, 1982).
16. Edgell, M.C.R. *Nature and Perception of the Bushfire Hazard in Southeastern Australia*, 8 (Monash University, Melbourne, 1973).
17. Wright, H.A. & Bailey, A.W. *Fire Ecology*, 274 (Wiley, New York, 1982).

18. McClement, F. *The Flaming Forest*, 46–47 (McClelland & Stewart, Toronto 1969).

19. Barrett, J.W. *Regional Silviculture of the United States* 2nd edn, 351 (1980).

● These two letters are a foretaste of a large amount of correspondence (on both sides) on this topic — Editor *Nature*.

The origins of malignancy

SIR — As an outsider, I would like to raise some questions about the distinctive features of malignant tissue.

One may suppose that the growth of normal tissue is regulated by the inhibition of cell division by some material produced by daughter cells. This mechanism suggests that cells produce substances which attach to receptor sites on the membrane of the cells involved, inhibiting cell growth and division; the inhibitors appear to be relatively tissue specific.

Accordingly, excessive growth in malignant tissue could be caused by a breakdown in this feedback mechanism. Although it is possible that malignant cells produce insufficient inhibitory substances to control their own growth, it seems more likely that the cells continue to produce these substances but are relatively unresponsive to them.

If the degree of specificity of the substances produced by malignant cells for the receptors of normal cells is sufficiently high, then the growth and division of normal tissue will be inhibited. As the malignant cells replicate in ever-increasing numbers, more and more growth inhibitory substances will be produced, overriding tissue specificities between different tissue types.

By extending this view of malignancy to its effect upon normal tissue, a number of clinical manifestations can be explained. First, benign tumours generally grow only by expansion and the surrounding compressed connective tissues tend to enclose the growth in a capsule. Malignant tumours, by contrast, grow also by infiltration and invasion. If malignant cells simply replicate at an enhanced rate, then expansion would occur along the lines of least resistance and physical restrictions would predominate. But if malignant cells also inhibit the growth and replication of the surrounding normal tissue, then invasion may be expected. Malignant cells would invade by inhibiting their competitors.

Second, malignant tissue has poorly developed blood vessels and bleeding occurs easily. Again this may be explained by the excessive concentration of growth inhibitory substances acting on normal tissue.

Third, advanced disseminated cancer is often accompanied by cachexia or generalized atrophy. When the malignancy is very extensive, then wasting of all normal tissues ensues.

Some cancers are typified by an extremely fast growth rate. Others which are equal-

ly damaging have a much lower growth rate. The extent of the damage to normal tissue may therefore not just be determined by the growth rate of a malignancy but also by the degree of general specificity of the substances produced by the tumour.

This model therefore seems capable of explaining some of the clinical manifestations of cancer. It suggests that malignant cells reduce the rate of growth and division of normal cells by producing substances which themselves play a normal part in the regulation of cell growth. It follows that removal or inhibition of these substances might convert a malignancy into the equivalent of a benign tumour, prevent the replacement of normal tissue and hence influence the course of the disease.

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Limits on the selfishness of DNA

SIR — Doolittle, Kirkwood and Dempster recently argued (*Nature* 307, 501; 1984) that some "selfish" DNAs (for example, elements which transpose duplicatively) may restrain their own reproduction within genomes to avoid driving their hosts, and hence themselves, into extinction. They suggested that such self-restraint should be expected to evolve in elements that impose a cost on their host's fitness which increases with copy number. That is, copy number should be limited so that host mortality does not exceed host reproduction. This view is fatally flawed, as it ignores a fundamental constraint on the evolution of "selfish" DNA.

Doolittle *et al.* presented a mathematical model which showed that a self-restrained element would be favoured over one with unlimited reproduction. They apparently did not realize that a potential host lacking either element would be favoured over hosts carrying one of the elements. An element which imposes *any* cost may be treated simply as a deleterious host gene, so selection of the host will eliminate that element. To persist, "selfish" DNA which is confined to one host (their model did not allow for transfer of the elements between hosts) must be beneficial to its host or be continuously generated *de novo* at a rate as great or greater than that of its loss due to selection (against its host) or vegetative segregation (failure to be vertically transmitted). The conclusion of Doolittle *et al.* may be reinterpreted to mean that self-restrained elements will be eliminated more slowly than unrestrained ones, but both will be eliminated. A different approach might show that self-restraint can evolve to avoid imposing *any* cost on the host, but modelling such a process might be difficult.

It has become common for biologists to imagine "selfish" DNA as a "genomic parasite", implying that it imposes a cost on its host, but most do not realize that true

parasitism would require a mechanism for horizontal transfer. Genetic elements which can be transferred horizontally may be able to offset a cost to their hosts by colonizing new hosts. This would require a high enough transfer rate to balance their losses by vegetative segregation and selection against their hosts. Temperate bacteriophage, some plasmids and temperate-like viruses of eukaryotes may fall into this category, but one would have to know rates of transfer and intensities of selection to make such a claim. A generalization is clear — one should expect genetic elements without a mode of horizontal transfer to be beneficial to their hosts and/or continuously generated *de novo* at rates sufficiently high to make up for their losses.

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Earthquake prediction from *b*-values

SIR — In 1981 W.D. Smith¹ made determinations of the *b*-value for a series of "moving windows" for various areas in New Zealand. The "*b*-value" is the slope (best estimate) of the relationship between magnitude and frequency of earthquakes over a fixed interval and fixed area; a "moving window" comprises a fixed number of events, say from event 1 to 50, the window being "moved" by shifting 10 events each time. Smith found that *b*-value maxima were associated with subsequent major earthquake events in accordance with a relationship he determined.

In 1982, with a postgraduate student whose supervision had been entrusted to me, Smith's principles were applied to Papua New Guinea. Excellent records of earthquake events are kept by the Papua New Guinea Geological Survey and were made available to us. Over a series of five years, it was possible to isolate the earthquake events associated with certain tectonic features and for some of these, to apply Smith's method. We found that the relationship he devised was similarly close for Papua New Guinea earthquakes.

This may be of interest to anyone wishing to study *b*-value maxima as earthquake precursors. The work is recorded in a university report². However, since the data covered 1,669 earthquake events, it was not practicable to publish it. It is not possible to predict earthquakes by this method from our work since the relationship is one between time after the *b*-value maximum and the magnitude of the subsequent event — the longer the time lapse, the greater the earthquake which follows. Furthermore, since then, Smith has had considerable doubts about the accuracy of the relationship he found (W.D. Smith, personal communication). However, it would be a pity if the data and findings, involving a considerable amount of work, should be unavailable to workers in the field, its only

record being in a form relatively obscure to library searchers.

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1. Smith, W.D. *Nature* 189, 135-139 (1981).
2. Emande, F.T. & Kiek, S.N. *Earthquake Precursors. Applications in Papua New Guinea* (Papua New Guinea University of Technology, Lac, 1982).

Brain, behaviour and the immune system

SIR — We read with great interest the News and Views article on "Psychoimmunology before its time" (*Nature* 309, 400; 1984), which calls attention to the now substantial evidence that brain and behavioural states can alter aspects of immune function. Attention is also called to other studies which have found links between similar behavioural states and health outcome. There has been a tendency, as noted in the article, to treat these two areas of research as if they were one and to confuse biological and epidemiological findings. *Nature's* near-scepticism concerning the explanatory power of the reported immune findings in relation to the role of stress in health outcome is well taken. But such an attitude might also be interpreted to suggest a sceptical approach to the biological findings themselves when they cannot adequately explain the epidemiological, such as with studies of bereavement. This type of reasoning would suffer from the same fault as that ascribed in the *Nature* article to the "psychoimmunologists" and further underscores the need to consider studies of behaviour and immune interactions in a biological context.

Current research on the effects of brain and behaviour on the immune system has provided reproducible, statistically and biologically significant data. The expanding knowledge about lymphocyte function and the developing technology may well provide the opportunity to elucidate further the association between brain and behavioural states and immunity. It remains to be determined whether such biological processes can explain aspects of physical illness.

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Somatic gene mutation and breast carcinoma (correction)

DUE to an editorial error, the above item of scientific correspondence (*Nature* 25 July, 310, 103-104; 1984) was incorrectly credited to a single author. It was in fact from M.A. Hulten, C.S. Rodgers and S.M. Hill, all at the Regional Cytogenetics Laboratory, East Birmingham Hospital, Birmingham B9 5ST, UK. □

Angular velocity: a new dimension in nuclei

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Nuclei can be studied from their ground states ($\sim 0 \hbar$) up to angular momenta of order $100 \hbar$, where they are literally pulled apart by centrifugal effects. This range of angular momenta can be viewed as resulting from 'cranking' the nucleus around a 'rotation' axis, where the critical variable is the cranking velocity. The calculated response of nuclei to such an imposed angular velocity corresponds well with recent observations, and includes a rich and varied interplay of collective and single-particle phenomena.

FIFTY years ago, with the discovery of the neutron, nuclear physics was at the forefront of studies of the ultimate constituents of matter and was where two of the four known fundamental interactions were first encountered. New forms of matter are still being sought at extremely high nuclear temperatures and densities, and attempts are being made to calculate nuclear properties from quantum chromodynamics, although such study has mainly separated off into elementary-particle physics. The nucleus is gradually being recognized as one of nature's most interesting quantal few-body systems. It concentrates into a single system many types of behaviour, almost all of which are found individually in other systems, which interact with one another in nuclei. A basic quantitative description of such inter-related phenomena is developing. The main thrust of nuclear physics has thus become understanding this quantal few-body system and its relationship to other such systems.

There are many approaches to the study of nuclear physics. The classical method of Rutherford¹ is still one of the most important: shoot a projectile at the nucleus and see what happens. Rutherford analysed the probability of scattering naturally-occurring α particles through various angles and deduced that the atom's positively charged nucleus was very small, 10,000 times smaller than the atom itself. Since that time nearly every conceivable projectile has been used: neutrinos, photons, electrons, mesons, nucleons and nuclei themselves, ranging from mass 2 (deuterium) to 238 (uranium). Depending on the projectile and bombarding energy, the result can be a very delicate transfer of a photon or nucleon, the complete fusion of target and projectile, or, at extremely high energies, an initial compression and heating of the target-projectile system followed by some kind of explosion. The analysis of 'what happens' has produced much of our knowledge of nuclear structure. Another approach is the study of the de-excitation of nuclei excited by one means or another, originally by natural radioactive decay. Generally, these nuclei decay from state to state by emitting photons until the ground state is reached—much like atomic de-excitation—although this depends on the excitation energy and, to some extent, the nucleus. The result of such studies is a mapping of the lowest energy levels of a nucleus, which is a major source of information about nuclear structure. Today, these methods are no longer distinct. The high-spin studies, for example, generally use a low-energy fusion reaction to bring large amounts of angular momentum into the fused system and then study the γ -ray decay of this system, which is usually completed in approximately a nanosecond.

The available information on nuclear structure is extensive and varied. Organizing it is inevitably somewhat subjective, but almost any scheme would have major divisions of 'single-particle behaviour' and 'collective behaviour', because the nucleus really is a 'few-body' (rather than a many-body) system and behaves accordingly. On one hand, many nuclear properties can be determined largely by the motion of a single nucleon, and a shell model works well; the nucleons are assumed to move

independently in their average potential, in close analogy to the atomic shell model. On the other hand, the nucleons, especially in heavier nuclei, often behave collectively, and there are close analogies both to solid-state and to familiar macroscopic systems. This behavioural dichotomy is the central theme of the high-spin studies we are interested in. But before describing these studies, we will briefly characterize these two aspects of nuclear behaviour.

The independent-particle description of nuclei has a long history. The discovery of the neutron² in 1931 immediately triggered the nuclear shell model, which had previously been tried unsuccessfully with protons, electrons and α -particles as constituents. It was proposed by Heisenberg³ and others who had just understood the structure of the atom, and indeed, the similarities of these two systems are striking. The difference amounts mainly to replacing the long-range Coulomb potential of the atom with a short-range one more appropriate for the nucleus. Initially, there were serious doubts about an independent-particle model for nuclei, and it was some time before it was fully appreciated that such a model works because the Pauli principle greatly restricts the possible scatterings, thereby increasing the mean free path. The next crucial idea was the large spin-orbit interaction ($l \cdot s$) in nuclei, proposed in 1949 by Mayer⁴ and by Haxel *et al.*⁵, which solved the problem that the early shell model had above mass 20. Because of the resulting large spin-orbit splitting, the highest j , orbitals drop down into the next lower shell and are called 'intruders'. They have different parity from their lower-shell neighbours, and thus they remain rather pure shell-model states and are easy to identify. These are the most important orbitals for building high angular momentum states. The (spherically symmetrical) shell model implies that the nuclear levels are bunched into groups constituting the shells. A filled shell has extra stability, so that near closed shells nuclei tend to be spherical, or nearly so. On the other hand, spherical shapes are disfavoured between closed shells. The nucleus then explores the shape degree of freedom to find a lower energy. Closed shells can also occur for nonspherical shapes with high symmetry, resulting in extra stability for such shapes at these particular nucleon numbers. Such seemingly random variations in nuclear properties (like the shape) due to the detailed shell structure are called shell effects.

One of the earliest collective phenomena observed in nuclei is fission, an evolution of nuclear shapes resulting in a division into two (or occasionally three) fragments. This process was discovered in 1939^{6,7}, with profound consequences. Not long thereafter (1947) the first of the normal vibrational modes of the nucleus was discovered⁸—the giant dipole resonance. This is an oscillation of neutrons against protons (isovector), and only recently has it been joined by some isoscalar giant resonances—oscillations of the nuclear shape (protons and neutrons in phase). Yet a third collective effect, rotation, turns out to be most important for the present discussion. Not all nuclei can rotate, but some of those that do have beautiful rotational level

structures, similar to (though less perfect than) those of molecules. All three of these collective effects are very well described by the liquid-drop model introduced by Bohr and Kalckar⁹ in 1937. This model considers the nucleus as a charged liquid drop, with volume, surface, Coulomb and (later) rotational energies. Combined with shell corrections, it gives the best existing estimates of the bulk nuclear properties. There is, however, at least one quantal collective effect in nuclei that is not described by the liquid-drop model, the pairing correlations. They are quite analogous to those in a superconductor or superfluid, and represent a coherent scattering of nucleon pairs among the levels near the Fermi surface. They have a role in high-spin studies; however, among the collective effects, it is rotation that dominates the high-spin phenomena.

Large angular velocities

The amount of angular momentum that a nucleus can hold is limited. The usual limitation is concerned with fission, and it is easy to understand that the centrifugal force associated with rotation will tend to encourage this process. Without any barrier, fission is rapid—occurring in around 10^{-20} s. Thus to 'exist' (that is, longer than $\sim 10^{-20}$ s) the nucleus must have a barrier against fission. Such barriers can be estimated using the liquid-drop model¹⁰, and the angular momenta at which they just vanish are shown in Fig. 1 by curve I_{II} as a function of nuclear mass, A (along the valley of β stability). The maximum is about $100 \hbar$ for $A \sim 130$. The curve falls off sharply at higher mass because of the increased Coulomb repulsion due to the additional protons. (Nuclei in the actinide region have measurable spontaneous fission lifetimes even at a spin of zero.) It falls off at lower mass because the nuclear moment of inertia (proportional to $A^{5/3}$) becomes smaller, so that for a given spin the rotational frequency is larger, thereby increasing the centrifugal force. If cold nuclei could be produced corresponding to the curve I_{II} , spectroscopic studies might be possible up to this limit of angular momentum. But the heavy-ion fusion reactions that bring in this much angular momentum to the compound nucleus also bring in several tens of MeV of excitation energy, greatly increasing the fission probability. To prevent fission, another process must successfully compete to de-excite the nucleus, and at such excitation energies this can only be particle evaporation. The time scale for this particle evaporation is 10^{-17} – 10^{-18} s, and to slow the fission down to such times, a fission barrier of the order of the neutron binding energy (~ 8 MeV) is required. The dashed line in Fig. 1 corresponds to an 8-MeV fission barrier, below which particle evaporation should dominate.

Figure 1 shows another interesting aspect. The curve I_{II} is determined by two different types of nuclear shape in different mass regions. At the highest masses the shape where the fission barrier just vanishes is spheroidal, more specifically, oblate. In fact, this is the stable shape everywhere below the curve I_1 . In the region between I_1 and I_{II} the equilibrium shape is ellipsoidal, generally triaxial, and elongates with increasing spin. These shapes are well known in the general context of equilibrium shapes of rotating objects. If one reverses the sign of the Coulomb energy term in the liquid-drop model, the equations describe a rotating gravitational object with surface tension (a liquid). The limit of negligible surface tension was applied to calculations of the shape of astronomical bodies (the first being the Earth) by Maclaurin¹¹ in 1742. The spheroidal shapes below I_1 in Fig. 1 bear his name, whereas the ellipsoidal ones above were found about 100 years later by Jacobi¹². If, on the other hand, one increases the surface energy greatly relative to the gravitational energy, one describes weightless rotating droplets (as examined by the Apollo astronauts using blobs of water). One can even consider negative masses (moments of inertia) and describe bubbles in ordinary fluids (or in nuclei, for that matter). The nuclear properties depicted in Fig. 1 are related to a very widespread type of behaviour. Since the dashed line in Fig. 1 includes some area above I_1 , there is a hope of finding some of these elongated shapes in nuclei at very high spins.

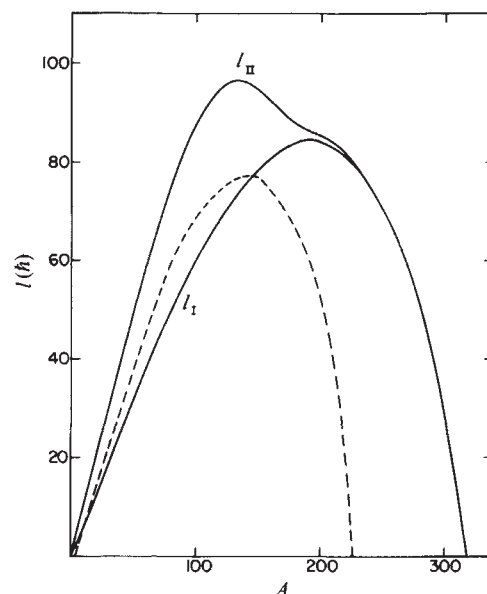


Fig. 1 A plot of angular momentum in a nucleus against the nuclear mass number, A . The curve I_{II} traces out the points where the fission barrier is zero, according to the liquid-drop model. Below the curve I_1 the equilibrium shape is an oblate spheroid (Maclaurin); between I_1 and I_{II} it is an ellipsoid, generally triaxial (Jacobi). The dashed line indicates a fission barrier of 8 MeV (from ref. 10).

Called 'superdeformed nuclei', they will be energetically favoured by shell effects for some nuclei and disfavoured for others. There is great interest in identifying such shapes at high spins, as they would give interesting information about the applicability of the liquid-drop model in these rather extreme conditions.

Figure 2 illustrates the decay modes for a nucleus of mass ~ 160 . The coordinates are nuclear excitation, E^* , and spin, I . The heavy lines divide the E^* – I space into regions of different decay modes. The 'yrast' line is the locus of states of lowest energy for a given spin, so that no states exist in the nucleus below this line. A typical heavy-ion fusion reaction might lead to an initial excitation energy of ~ 65 MeV and spin distribution ranging from 0 to $\sim 65 \hbar$ as indicated by the light line in Fig. 2. As long as the nucleus has sufficient energy above the yrast line to emit nucleons (~ 10 MeV), it usually does so; γ -ray emission is too slow to compete well with particle evaporation. But at excitations below the nucleon binding energy, γ -ray emission takes over and de-excites the nucleus to its ground state. The angular momentum removed by the particle evaporation is small if neutrons or protons are involved ($\sim 1 \hbar$ per particle and only a few particles). Figure 2 shows that the highest spins (longest γ -ray cascades) will be associated with the fewest neutrons emitted. These γ -ray cascades have two principal types of transitions. The 'statistical' transitions carry off energy but little angular momentum and so cool the nucleus towards the yrast line. The yrast-like transitions follow paths roughly parallel to the yrast line and remove the angular momentum of the system. These are often collective rotational transitions. There are many pathways from the beginnings of a high-spin γ cascade until a region near the yrast line is reached, with the result that no single transition has enough intensity to stand up in the spectrum (with present techniques). This is the origin of the name 'continuum' γ -ray spectrum, although this is not a true continuum. When the nucleus has cooled sufficiently, the population condenses into a few pathways and the transitions in these pathways stand up in the spectrum and are resolved. This typically happens in the spin range 30 – $40 \hbar$ for masses around 160, and it provides a logical division in high-spin studies. In the lower-spin region,

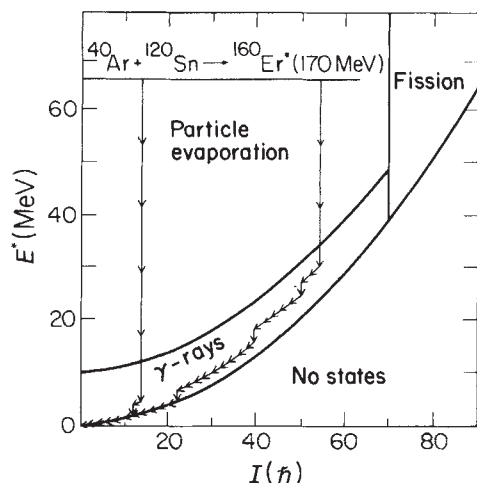


Fig. 2 The heavy lines constitute a phase-like diagram for the decay modes of a nucleus having mass number about 160 as a function of excitation energy and angular momentum. The lighter horizontal line indicates the range of angular momentum brought in by a typical heavy-ion reaction, following which two of the many possible decay pathways are shown (longer arrows represent neutron evaporations and shorter ones γ -ray emissions).

one can use all the techniques of conventional γ -ray spectroscopy and develop detailed information on the nature of the transitions and states involved. At the higher spins where the population is spread out too much to permit the study of individual transitions, new techniques are providing a picture of the average nuclear behaviour. Intensive work is, of course, under way to resolve this 'continuum' spectrum using new detector systems.

Shape effects

One of the most important factors in determining the physics of high-spin states is illustrated by rotational behaviour of rigid classical objects. In Fig. 3, the moment of inertia of such an object (solid lines) is compared with that of a rigid sphere for a variety of shapes and rotational axes. The shape and axis is defined by γ , which varies from -120° to 60° as the object varies from a prolate shape rotating about its symmetry axis, through oblate and prolate shapes rotating about axes perpendicular to the symmetry axis, to an oblate shape rotating about its symmetry axis. These axially symmetrical shapes are shown in Fig. 3, and the regions between correspond to shapes with all three axes different—triaxial. The deformation is given in terms of ϵ , which is to lowest order just $\Delta R/R$, the difference in radii divided by the average radius. Values of ϵ between 0.2 and 0.3 are typical for the familiar deformed rare-earth and actinide nuclei, and 0.6 corresponds to an axis ratio of 2:1, the largest known in nuclei. The largest moments of inertia, and therefore the lowest rotational energies, occur for the range of shapes between $\gamma = 0^\circ$ and 60° . The very largest moment of inertia for moderate deformations is for $\gamma = 60^\circ$, an oblate shape rotating around its symmetry axis, and it is for this reason the Earth has such a shape. The full liquid-drop model treatment of a rotating nucleus¹⁰ including surface and Coulomb energies in addition to these geometrical shape considerations is shown by the dots in Fig. 3. There is no strong shape preference in these additional liquid-drop model terms, so that simple geometry determines the liquid-drop shapes. This is significant, as the liquid-drop model is our best guide to such macroscopic nuclear properties and is even the average limit to which some of the microscopic (individual particle) models are normalized.

To see how important these geometrical shape effects are, one must choose a mass and spin, and for $A = 160$ and $I = 60$, an energy scale is given on the right side of Fig. 3. The variation

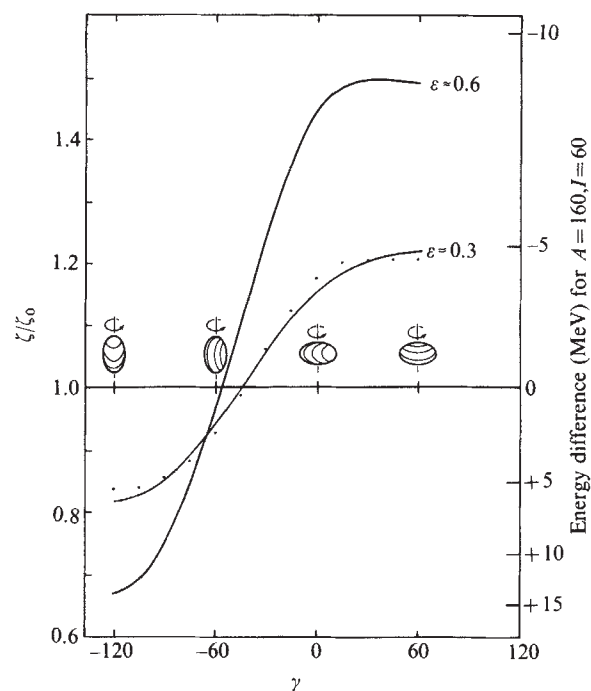


Fig. 3 The ratio of the moment of inertia of a rigid ellipsoid to that of a rigid sphere as a function of the ellipsoidal shape parameter, γ . The two curves are for deformations ($\sim \Delta R/R$) of $\epsilon = 0.3$ and 0.6 . The parameter γ defines a rotation axis, as well as a shape, and the four axially-symmetrical shapes (two prolate and two oblate) are shown by the small drawings. The scale on the right converts the moment-of-inertia ratios into energy differences for a mass number around 160 and a spin of $60\hbar$. The dots indicate the energy differences from the full liquid-drop model (surface and Coulomb energies in addition to the rotational energy implied by the moment of inertia).

for $\epsilon = 0.3$ of about 10 MeV is larger than typical shell effects (~ 3 MeV) so that for this spin the shape effects considered here should be dominant. However, the rotational energy varies as I^2 so that, for $I = 30$, the shell effects and these classical shape effects should be about equivalent, and below $I \sim 20$ the shell effects will dominate. The arguments made here would seem to apply only for collective nuclear rotations, and even then only if the nuclear moment of inertia has the rigid-body value, neither of which is obviously the case. In fact, it is generally believed that rotating nuclei at high spins will, on average, have the rigid-body moment of inertia, and this is the case for independent particle motion in a rotating anisotropic harmonic-oscillator potential¹³. (The smaller moments of inertia observed in nuclei at low spins are due largely to the pairing correlations, which should be quenched by the Coriolis force above $\sim 30\hbar$). Furthermore, even in noncollective cases, the trajectory of lowest levels (for a Fermi gas) follows that given by rotating the appropriately shaped rigid body¹⁴. Thus, these geometrical arguments are expected to be valid, and shapes in the $\gamma = 0$ – 60° range should dominate at high spin, that is above $\sim 30\hbar$ in the $A = 160$ region. There are several detailed microscopic calculations that agree with these expectations.

There is a further important aspect to these shapes. The rotation of a nucleus about an axis perpendicular to the symmetry axis is a collective rotation with smooth bands, $E(I) \propto I(I+1)$, and strongly enhanced $E2$ transitions connecting the levels. There are many examples of such rotors in the region around mass 160, one of which is shown in the left-hand side of Fig. 4. This is the lowest-lying sequence of levels in ^{158}Er . The odd spins are missing, indicating that the two ends of the nucleus are indistinguishable (symmetry under rotation of 180° about the x or y axis—like homonuclear diatomic molecules).

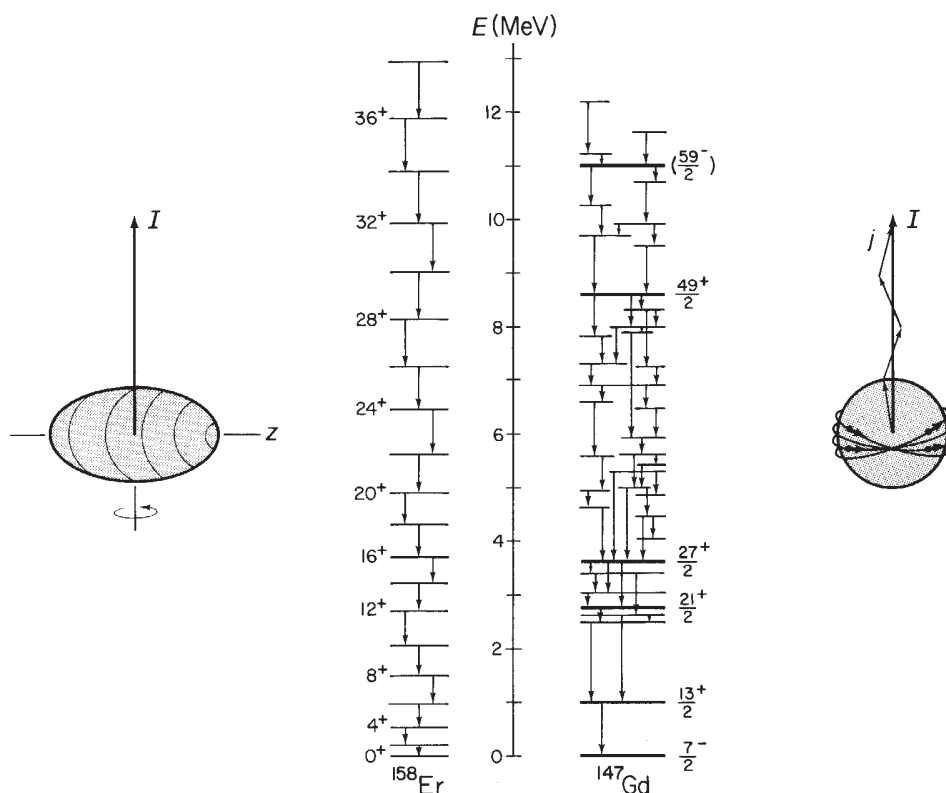


Fig. 4 The level schemes are for the lowest-energy high-spin states in ^{158}Er and ^{147}Gd (refs 31–33). The sketches on the left- and right-hand sides illustrate the dominant source of angular momentum in each case, collective rotation and single-particle alignment, respectively.

Also the electric quadrupole transitions connecting the levels generally vary smoothly in energy and are enhanced by about 150 times over that expected for a single proton, indicating a collective quadrupole shape as shown on Fig. 4. On the other hand, a quantal system like the nucleus cannot rotate around a symmetry axis. This degree of freedom is contained in the single-particle motions. Thus, a nucleus with $\gamma = 60^\circ$ builds up its angular momentum by aligning that of one or more individual nucleons with the symmetry axis, like spherical closed-shell nuclei. An example of this behaviour is shown on the right-hand side of Fig. 4. The ^{147}Gd nucleus is nearly spherical in its ground state and builds its angular momentum by aligning particles as shown on the far right of Fig. 4. The aligned particles give the system an oblate shape that effectively rotates about its symmetry axis, $\gamma = 60^\circ$. The motion is almost completely noncollective and the transitions in the ^{147}Gd scheme are quite irregular in energy and are not enhanced. Most nuclei combine these types of behaviour, leading to triaxial shapes between 0° and 60° . This tendency can already be seen in the ^{158}Er scheme, where there are irregularities around spins 16 and 26, which correspond to single-particle alignments.

Rotational alignment

The lack of smoothness in rotational spectra came as a surprise when in 1971, a discontinuity was found in the energies of the ground-state rotational bands of several rare-earth nuclei¹⁵. As the nucleus de-excites from a high initial spin, the regular increase in rotational period (slowing down) is interrupted occasionally by rather sizeable decreases. These correspond to internal rearrangements, 'nuclearquakes', and are generally called 'backbends'. They are related to the pairing correlations in nuclei.

The nuclear pairing correlations have an important role up to spins around $30\hbar$. The nucleon orbitals in a static deformed potential are two-fold degenerate, corresponding to a time reversal of their motion. The angular momentum, j , of the nucleon has projections, $\pm\Omega$, along the symmetry axis and, when occupied by two nucleons, results in total angular momen-

tum zero. Every orbital, characterized by j, Ω , can give rise to such a spin-zero pair. The nucleons in a filled orbital near the Fermi level can scatter as a pair into a nearby empty orbital, and the coherent scattering pattern that develops comprises the nuclear pairing correlations. This smearing of the Fermi level blurs the distinction between particle and hole states over the region of the scattering, resulting in 'quasiparticles' that are mixtures of the two. It is interesting that these correlations are closely analogous to those in superconductors or superfluids. In fact, the equations of Bardeen, Cooper and Schrieffer¹⁶ (BCS) that first explained superconductivity are taken over exactly into the nuclear case^{17–19} and give nearly correctly both the systematic mass difference between even-even nuclei (all paired, zero quasiparticles) and odd-mass nuclei (one quasiparticle) and the ~ 2 -MeV level gap in even-even nuclei (zero to two quasiparticle energy).

These pairing correlations affect the ability of the nucleus to generate angular momentum. This is plausible since, insofar as the pairs are coupled to spin zero, they can contribute nothing towards the angular momentum. This causes a factor of two or three reduction in the nuclear moment of inertia, which is given reasonably well by the BCS wave functions. It follows that angular momentum will tend to weaken the pairing correlations, thus increasing the moment of inertia and reducing the rotational energy. The mechanism of this weakening is the Coriolis force, which acts oppositely on the two members of the pair, lifting their degeneracy. Ultimately, the Coriolis force will tend to align the particle angular momentum as well as possible with the rotation axis. This process is analogous to the effect of a magnetic field on the paired electrons in a superconductor, where there is a sudden change back to the normal state when a critical magnetic field is reached. At first, it was thought that a nucleus might behave similarly when a critical angular momentum is reached, but the nucleus differs from the superconductor in at least two respects: (1) rather than approximately Avogadro's number of electrons, there are ~ 100 nucleons in the nucleus, of which only 10–20% are near the Fermi level and thus participating in the pairing correlations, and (2) the nucleons have a wide spread of j values ranging from $1/2$ to $\sim 13/2$ (for mass

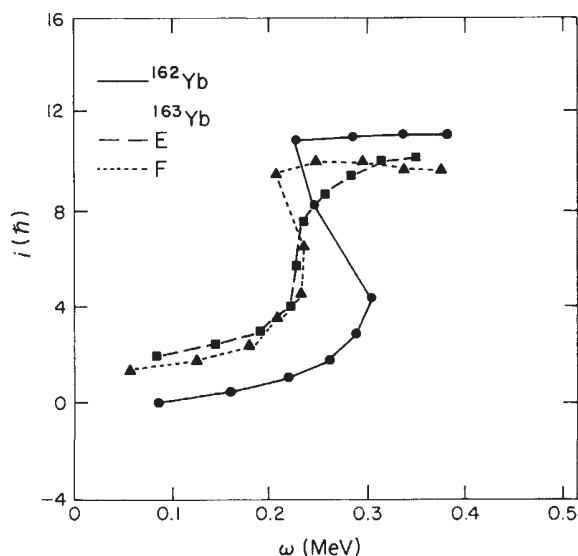


Fig. 5 The aligned angular momentum, i , is plotted against rotational frequency, ω , for the first backband ($i_{13/2}$ alignment) region of the lowest-lying (yrast) sequence in ^{162}Yb and of two bands in ^{163}Yb (labelled E and F). The midpoint of the sharp rise is approximately the crossing frequency (from B. Herskind, personal communication).

~ 100). The result is that the nuclear phase transition is not sharp but broad, as evidenced by a gradual rise in the moment of inertia for spins up to ~ 20 – $30 \hbar$. However, within this gradual rise there is occasionally a large irregularity that corresponds to the rather complete alignment of a particular pair of high- j nucleons. This comes about because the Coriolis force is proportional to j and thus affects high- j particles most strongly, so that at some point the nucleus finds it energetically most favourable to align such a pair almost completely, while keeping the pairing correlations among the lower- j nucleons. This is what causes the nuclear quakes. In ^{158}Er and many other nuclei of that region, it is the sudden alignment of a pair of $i_{13/2}$ ($l=6$, $s=+1/2$) neutrons²⁰ that causes the large irregularity at frequencies around 0.25 MeV ($I \sim 16$). Some of these same nuclei suffer a second smaller discontinuity when a pair of $h_{11/2}$ protons aligns at frequencies around 0.4 MeV ($I \sim 26$). Such detailed information is not available at frequencies much higher than this. However, this rotation alignment of high- j nucleons gives much more information about nuclear structure than might be apparent from the discussion so far.

There is a point of view that angular velocity or frequency is an important dimension along which nuclear properties can be measured. (The canonical expression for the frequency of a rotor is: $\hbar\omega = dE/dI$, and since the typical $E2$ rotational transition carries two units of spin, $\hbar\omega$ is approximately half the rotational transition energy. This is fortunate as the transition energy is one of the most easily observable quantities.) Use of this intensive variable seems likely to provide clearer views of the underlying physics than would result from use of the extensive variable, angular momentum, even though the latter is the quantized and conserved quantity. In this picture a rotational band is just a sequence of snapshots of the same configuration at increasing rotational frequency, in which different properties can be observed. The alignments discussed above stand out in the various bands like mileposts, and we now concentrate on these and, further, on the critical frequency, $\hbar\omega_c$, at which they occur. Much of the available information is on the alignment of two $i_{13/2}$ neutrons in nuclei around mass 160. In Fig. 5 the aligned angular momentum, i , for this pair (measured by the difference in angular momentum between the band under con-

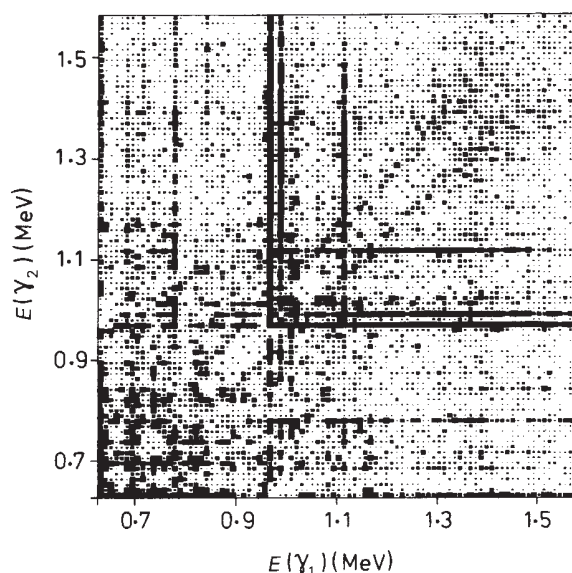


Fig. 6 A plot showing the $E(\gamma_1) - E(\gamma_2)$ correlations in ^{152}Dy following the reaction of 205 MeV ^{48}Ca on ^{108}Pd . The data were taken with Compton-suppressed Ge detectors, have been corrected for the response function and efficiency of the detectors, and have had uncorrelated events subtracted by an iterative procedure (from ref. 24).

sideration and a reference band) is plotted against rotational frequency for three bands. The solid line is for the lowest-energy band in the even-even nucleus ^{162}Yb , which is much the same as the band in ^{158}Er plotted in Fig. 4. The critical frequency is about 0.26 MeV and the aligned angular momentum is $\sim 10 \hbar$ ($12 \hbar$ is the maximum for two $i_{13/2}$ neutrons). The dashed lines are for two bands in the nucleus ^{163}Yb with one additional neutron located in an orbital labelled either E or F. These orbitals comprise a time-reversed pair at zero rotational frequency and are not very pure shell-model states, although their dominant component is $h_{9/2}$. In the even-even nucleus ^{162}Yb , this pair of states (E, F) is available for the pairing correlations, and, in particular, a pair of $i_{13/2}$ neutrons can scatter into it. On the other hand, in ^{163}Yb it is blocked by the odd nucleon for the bands based on either E or F. The pairing correlations are thereby weaker in general, and, in particular, for a pair of $i_{13/2}$ neutrons. It is then easier to unpair and align the $i_{13/2}$ neutrons, and this occurs at a lower rotational frequency, ~ 0.22 MeV, as seen in Fig. 5. The shift is clear, closely reproducible in other nearby nuclei²¹, and can be related through calculations to the change in the pairing correlations involved; it turns out to correspond to a 20–30% reduction in pairing. Such properties are calculated by cranking a deformed shell-model potential around an axis perpendicular to the symmetry axis and can relate a frequency shift to a change in the pairing gap with reasonable confidence. Thus, blocking just one orbital near the Fermi level reduces the pairing correlations appreciably, a result that is confirmed by other kinds of experiments—transfers of pairs of nucleons, and directly from the odd-even mass difference. The pairing correlations in nuclei are marginal, and three or four blocked levels of either type (protons or neutrons) are enough to destroy the correlations for that nucleon type. But the analysis of data like those shown in Fig. 5 can be carried considerably further.

The discussion so far has involved blocking one particular pair of orbitals, E and F. Others can be blocked, and most of the calculations of nuclear pairing effects assume identical results for blocking any orbital equally distant from the Fermi level (called 'monopole pairing'). This is, in fact, not very reasonable, since the aligning neutrons in this case are $i_{13/2}$,

with a specific orientation relative to the symmetry axis, and some orbitals will have better spatial overlap with these than others. It seems that the more similar ones will have a greater effect on the pairing. One measure of the shape of an orbit is its quadrupole moment relative to the nuclear symmetry axis, and, the magnitude of the frequency shift has been shown²² to be correlated with the similarity of the quadrupole moment of the blocked state to that of the aligning particles. In fact, blocking a very differently shaped orbital produced no shift in $\hbar\omega_c$. Such higher order effects are referred to as 'quadrupole pairing'. Their appearance results from the few-body nature of the nucleus, and gives us some information about pairing phenomena which do not occur in macroscopic systems such as superconductors.

Exploitation of the rotational-frequency dimension has just begun. Studies of the type outlined above can be extended to: (1) additional blocked orbitals; and (2) other aligning pairs. Figure 5 shows that the amount of aligned angular momentum, i , varies between the even (0–2 quasiparticle) and the odd (1–3 quasiparticle) systems. This is probably also a pairing effect but is not yet as well understood as the critical frequency effects discussed above. There are many other properties to study as a function of frequency, the detailed effects of (usually small) changes in the nuclear shape being another one of interest. The process of quenching the pairing correlations is also being studied. It has not yet been possible to obtain quantitative measures of this quenching, although it is clearly large in some cases.

Highest spins

Above about 30 or 40 $\hbar$, individual transitions cannot at present be resolved following heavy-ion fusion reactions because too many de-excitation pathways exist. Thus, to study higher spins we must study unresolved γ -ray spectra. Most of the techniques devised to do this have involved measuring average moments of inertia, and considerable progress has been made.

As a consequence of the interplay between collective and single-particle motions, there are a variety of moments of inertia one can measure and compare with detailed nuclear model calculations. The first distinction to make is between kinematic and dynamic values. The equation for the rotational energies of a symmetrical top is:

$$E(I) = \frac{\hbar^2}{2\mathcal{I}} I(I+1) \quad (1)$$

where $\mathcal{I}$ is the moment of inertia. One sees that a moment of inertia may be defined from the first derivative of the energy with respect to spin:

$$\frac{\mathcal{I}^{(1)}}{\hbar^2} = I \left(\frac{dE}{dI} \right)^{-1} = \frac{I}{\hbar\omega} \quad (2)$$

where $\mathcal{I}^{(1)}$ is called the 'kinematic' moment of inertia because it concerns the motion of the system—the ratio of angular momentum to angular frequency. The second derivative leads to a definition:

$$\frac{\mathcal{I}^{(2)}}{\hbar^2} = \left(\frac{d^2E}{dI^2} \right)^{-1} = \frac{dI}{\hbar d\omega} \quad (3)$$

where $\mathcal{I}^{(2)}$ is called the 'dynamic' moment of inertia because it concerns the way that the system will respond to a force. If there is only the kinetic energy term as given in equation (1), these are equal, but, in general, when there are additional I -dependent terms in the hamiltonian these two moments of inertia will differ. In the present case, the Coriolis force perturbs the internal nuclear structure, giving rise, in lowest order, to an $(I \cdot j)$ term, so that $\mathcal{I}^{(1)} \neq \mathcal{I}^{(2)}$. This situation is not uncommon in other branches of physics. The arguments carry over into translational motion, where $p^2/2m$ is analogous to $I^2/2\mathcal{I}$, and additional momentum-dependent terms in the hamiltonian give rise to two observed masses. An electron moving in a crystal lattice is a close analogue²³, where the kinematic mass deter-

mines the level density and related statistical mechanical properties, whereas the response of the electron to an external force depends on a different, dynamic mass. In cases where the extra (angular) momentum-dependent term(s) depend on $(I^2)p^2$ (or as long as they can be expanded in lowest order as such), they can be taken together with the kinetic term to give a renormalized (moment of inertia) mass.

These two moments of inertia can be defined in principle for any sequence of states desired, but certain ones occur naturally in the decay processes. If the particle configuration is frozen, so that one is confined to a collective rotational band, the appropriate moments of inertia are $\mathcal{I}_{\text{band}}^{(1)}$ and $\mathcal{I}_{\text{band}}^{(2)}$. When there is no perturbation (alignment, shape change, and so on) of the internal structure along this band, these correspond to the true 'collective' values, and this is an approximation often made. In general, however, a single decay pathway involves a sequence of bands having different alignments or shapes. It is then natural to define effective moments of inertia $\mathcal{I}_{\text{eff}}^{(1)}$ and $\mathcal{I}_{\text{eff}}^{(2)}$, which include both the collective contribution and contributions caused by changes in particle alignment and shape. For the unresolved spectra from the highest spin states, the population is spread over many bands in many decay sequences. Nevertheless, average values for these moments of inertia can be determined in the following ways.

The γ -ray spectrum from a rotational nucleus is highly correlated in time, spatial distribution and energy. For a perfect rotor, this spectrum is composed of equally spaced lines, up to some maximum energy corresponding to the decay of the state with highest angular momentum. One aspect of this distribution is that no two γ rays have the same energy. If plotted on a two-dimensional diagram of $E_\gamma(1)$ against $E_\gamma(2)$, such energies give a pattern with no points along the diagonal and a series of ridges parallel to it. The width of the valley along the diagonal is determined by the difference between rotational γ -ray energies and thus gives values for $\mathcal{I}_{\text{band}}^{(2)}$. The important point is that the spectrum need not be resolved to determine the valley width. All that is required is that the populated bands have similar moments of inertia at a given frequency (γ -ray energy).

The data²⁴ in Fig. 6 come mainly from ^{152}Dy formed by bombarding ^{108}Pd with ^{48}Ca at sufficient energy (205 MeV) to bring into the fused system all the angular momentum the nucleus can hold ($\sim 70 \hbar$). The data have been corrected for the response function and efficiency of the Ge detectors, 'symmetrized' around the diagonal to improve the statistics, and have an 'uncorrelated' background subtracted. A valley is reasonably clear between 0.8 and 1.35 MeV. (There are also horizontal and vertical lines corresponding to coincidences with strong discrete γ -ray lines from states below 40 $\hbar$.) The width of the valley is roughly constant and can be evaluated to give $\mathcal{I}_{\text{band}}^{(2)}/\hbar^2 \approx (85 \pm 2) \text{ MeV}^{-1}$, around 1.4 times larger than the rigid-sphere value. Such a large band moment of inertia probably implies a large deformation—close to an axis ratio 2:1. Thus this spectrum provides the best evidence to date for superdeformed shapes at high spins (0.8–1.35 MeV suggests a spin range $35 < I < 60$), and, indeed, the microscopic calculations indicate a strong shell effect favouring such shapes in the region of ^{152}Dy . This is a powerful technique for identifying rotational bands in an unresolved spectrum, and will, no doubt, contribute greatly to our knowledge of the highest spin states.

The effective moments of inertia are simpler in some respects. They involve relating a collective γ -ray energy with a spin or measuring the number of γ rays in an energy interval. The former gives $\mathcal{I}_{\text{eff}}^{(1)}$ values that have been measured in several different ways, originally by relating the maximum γ -ray energy in a spectrum to the estimated maximum spin input²⁵. However, $\mathcal{I}_{\text{eff}}^{(2)}$ is much more sensitive to the nuclear structure. It is possible to measure $\mathcal{I}_{\text{eff}}^{(2)}$ because, in a spectrum consisting only of 'stretched' electric quadrupole ($I \rightarrow I-2$) transitions (which is a good approximation in regions of rotational behaviour), the number of transitions in a given γ -ray energy interval is half the spin removed by that interval. If one knows the fraction of the observed population that goes through the interval, then the

height of the spectrum gives directly $\mathcal{J}_{\text{eff}}^{(2)}(\omega)$. A method has recently been developed²⁶ to estimate the feeding as a function of spin, and $\mathcal{J}_{\text{eff}}^{(2)}$ values have been measured up to spins around $60\hbar$ in the mass 160 region. With increasing frequency these generally show broad and rather smooth variations from below the rigid-sphere value to as much as 1.6 times larger. This could be due to variations in deformation, as was discussed for $\mathcal{J}_{\text{band}}^{(2)}$ values, but $\mathcal{J}_{\text{eff}}^{(2)}$ contains, in addition, single-particle contributions and these are subject to rather large shell effects. Measurements of $\mathcal{J}_{\text{band}}^{(2)}$ by the correlation method in some of these nuclei show values much smaller than $\mathcal{J}_{\text{eff}}^{(2)}$ at the highest spins, arguing against superdeformation as the cause of the large $\mathcal{J}_{\text{eff}}^{(2)}$ values. Calculations support the conclusion that in nuclei heavier than ^{152}Dy one should see single-particle effects from the next higher major shell before onset of superdeformation.

While such data do not give the detail obtained at lower spins from studies of resolved lines, they are, nevertheless, beginning to give important insights into the physics of these highest spin states. No doubt techniques to study unresolved spectra will develop further if we do not learn how to resolve this continuum spectrum.

The future

The study of nuclei at high spin is an active area. There are new types of detector systems coming into operation, as well as a rapid development of the theoretical tools to interpret the data and to serve as a guide in further data acquisition.

The information on high-spin states comes mostly from studies of γ rays; thus there is a strong incentive to develop more powerful γ -ray detector systems. An obvious goal is to measure the energy and angle of every γ ray emitted from a decaying high-spin state (up to 35 transitions in some cases). Two 4π detector systems have been built to accomplish this. These instruments are both shells of NaI about 8 inches in inner radius and 7 inches thick (insuring almost complete absorption of all γ rays). To isolate the individual γ ray, the shell is divided into many elements, 72 for the system built at Oak Ridge²⁷ and 162 for the one in Heidelberg²⁸. These instruments can measure the number of γ rays and the total γ -ray energy emitted, each with about 20% resolution (FWHM). In general, for the decay of high-spin states the number of γ rays is related to the initial spin and the total energy to the initial excitation energy. Thus these instruments can isolate a rather small initial population region, as can be visualized in Fig. 2. The spectrum of γ rays from this limited region should be much simpler to study than that from the entire reaction, perhaps even fully resolvable. Furthermore, the spin and/or excitation energy (nuclear temperature) of the region can be altered by changes in the gating conditions. Such possibilities are exciting and, already, out of some initial studies²⁹, evidence for variations in the γ -ray spectrum with initial temperature has been found.

These 'crystal balls' also measure the energy and angle of (nearly) every γ ray. This will surely be interesting to explore, but is not quite as powerful as it might be because the γ -ray energy resolution in NaI is rather poor, 5–6% (FWHM) for a 1-MeV γ ray. In fact, these crystal balls will probably often be used in conjunction with detectors of higher energy resolution, mainly germanium semiconductor detectors, which give about 0.2% resolution at 1 MeV (but cannot be made so large). Such considerations have led to 'combination' detector systems; a low-resolution highly efficient shell to give the initial-state selection (as described above) coupled with an array of high-resolution Ge detectors. One such apparatus exists at Daresbury³⁰, another is nearly complete at Berkeley, and several others are under construction. Such systems will contribute both towards resolving the continuum and to the detailed spectroscopy of the lower spin regions.

The high-spin field is, thus, giving rise to a new generation of data acquisition systems. Fortunately, the theoretical developments have kept pace. Single-particle motion can be calculated in deformed potentials of several types, and, further, these can be cranked about various axes to simulate the rotation. Such calculations, though still approximate, can be an excellent guide. Virtually all the properties now measured can also be calculated, and comparison provides a stimulus both to interpreting the experimental data and to improving the calculations. In the lower spin regions the pairing-correlation studies are an example of this process.

Many questions remain. Two in the lower-spin region are: where and how are the pairing correlations finally quenched by the rotation; and what is the detailed nuclear shape and how does it vary for different bands (configurations), as a function of spin in a single band, and as a function of nuclear temperature. At higher spins the questions are broader. What are the appropriate quantum numbers to describe the system? This is related to the question of whether there are still large single-particle effects (irregularities) or whether the nucleus has been homogenized at the highest spins and the motion has become fully collective. One is examining in detail here the generation of collective properties out of an underlying single-particle structure. Then there are the interesting questions connected with 'superdeformations' and the shape evolution connected with fission. Over a broad range of masses, the angular momentum we can study is limited mainly by fission, so it is clear we can reach situations where the centrifugal force produces major changes. How these are influenced by shell effects or other aspects of the single-particle motion will be fascinating to study. The nucleus provides a chance to study a few-body quantal system over a broad range of a variable, the rotational frequency. The results should enrich our perspectives on such systems.

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1. Rutherford, E. *Phil. Mag.* **21**, 669–688 (1911).
2. Chadwick, J. *Proc. R. Soc. A* **136**, 692–708 (1932).
3. Heisenberg, W. *Z. Phys.* **77**, 1–11 (1932).
4. Mayer, M. G. *Phys. Rev.* **75**, 1969–1970 (1949).
5. Haxel, O., Jensen, J. H. D. & Suess, H. E. *Phys. Rev.* **75**, 1766 (1949).
6. Hahn, O. & Strassman, F. *Naturwissenschaften* **27**, 11–15 (1939).
7. Meitner, L. & Frisch, O. R. *Nature* **143**, 239–240 (1939).
8. Baldwin, J. C. & Klaiber, G. S. *Phys. Rev.* **71**, 3–10 (1947).
9. Bohr, N. & Kalckar, F. K. *danske Vidensk. Selsk. Mat-fys. Medd.* **14**, 10–40 (1937).
10. Cohen, S., Pislis, F. & Swiatecki, W. J. *Ann. Phys.* **82**, 557–596 (1974).
11. Maclaurin, C. *A Treatise on Fluxions* (Ruddimans, Edinburgh, 1742).
12. Jacobi, C. G. J. *Annaln. Phy. Chem.* **33**, 229 (1834).
13. Bohr, A. & Mottelson, B. R. *Mat. Fys. Medd. Dan. Vid. Selsk.* **30**, No. 2 (1955).
14. Bohr, A. & Mottelson, B. R. *Nuclear Structure*, Vol. 2, 80 (Benjamin, Reading, 1975).
15. Johnson, A., Ryde, H. & Sztarkier, J. *Phys. Lett.* **34B**, 605–608 (1971).
16. Bardeen, J., Cooper, L. N. & Schrieffer, J. R. *Phys. Rev.* **106**, 162–164 (1957); **108**, 1175–1204 (1957).
17. Bohr, A., Mottelson, B. R. & Pines, D. *Phys. Rev.* **110**, 936–938 (1958).
18. Belyaev, S. T. *Mat. Fys. Medd. Dan. Vid. Selsk.* **31**, No. 11 (1959).
19. Migdal, A. B. *Nucl. Phys.* **13**, 655–674 (1959).
20. Stephens, F. S. & Simon, R. S. *Nucl. Phys. A* **183**, 257–284 (1972).
21. J. D. Garrett, *Phys. Rev. Lett.* **47**, 75–78 (1981).
22. Garrett, J. D., Hagemann, G. B. & Herskind, B. *Nucl. Phys. A* **400**, 113c–129c (1983).
23. Bohr, A. & Mottelson, B. R. *Phys. Scripta* **24**, 71–76 (1981).
24. Nyako, B. M. *et al. Phys. Rev. Lett.* **52**, 507–510 (1984).
25. Simon, R. S., Banaschik, M. V., Diamond, R. M., Newton, J. O. & Stephens, F. S. *Nucl. Phys. A* **290**, 253–271 (1977).
26. Deleplanque, M. A. *et al. Phys. Rev. Lett.* **50**, 409–412 (1983).
27. Jaaskelainen, M. *et al. Nucl. Instrum. Meth.* **204**, 385–405 (1983).
28. Metag, V. *Proc. Geiger Memorial Meet.* (Springer, Berlin, 1983).
29. Lee, I. Y. *et al. Proc. Conf. on High Angular Momentum Properties of Nuclei* (ed. Johnson, N. R.) 339 (Harwood Academic, New York, 1983).
30. Twin, P. J. *et al. Nucl. Phys. A* **409**, 343c–351c (1983).
31. Burde, J. *et al. Phys. Rev. Lett.* **48**, 530–533 (1982).
32. Kleinheinz, P. *et al. Z. Phys. A* **290**, 279–295 (1979).
33. Chowdhury, P. *et al. Workshop on Nuclear Structure at High Spin*, Risø (Niels Bohr Institute, 1983).

Evidence for an increasing 59-s period in the X-ray emission from Geminga

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Einstein Observatory and Exosat X-ray data from 1E0630+178 reveal a $\sim 50\%$ periodic emission at ≥ 59 s. This confirms the earlier SAS 2 and COS B findings on 2CG195+4 at $E > 50$ MeV, and provides the temporal signature for the identification of Geminga. The observations, spanning over a decade (1973–83), also suggest a very high positive period derivative, $\dot{P}$, which has become still higher between 1979 and 1983. A simple source scenario is proposed, based on a binary system containing a compact object responsible for the high energy emission, and also taking into account recent ultra high energy γ -ray measurements.

THE high-energy (>50 MeV) γ -ray source 2CG195+4 (Geminga), discovered by the SAS 2 satellite^{1,2} and confirmed by COS B (see ref. 3), is the brightest of the unidentified γ -ray sources, and the one which has the smallest 'error box', with a radius of $\sim 0.4^\circ$. As such, it has attracted many searches for counterparts at other wavelengths (see ref. 4 for a review); recently, a particularly interesting new Einstein X-ray source, and its optical counterpart candidate (ref. 5), has been proposed⁶ as the object responsible for the γ -ray emission.

We have analysed the Einstein X-ray data as well as the new Exosat observation of this source⁷, and discovered a ≥ 59 s periodicity with a very high value of the period derivative $\dot{P}$. Such periodicity was originally reported by the SAS-2 team², and was later confirmed⁸ and then retracted⁹ by the COS B collaboration as non-statistically significant.

It now seems that the 'Lamb–Thompson' method of analysing the 121 SAS 2 photons, albeit crude, was indeed correct and, more important, that the timing signature confirms the identification of Geminga.

Other interesting properties of this object are revealed when all the published data are taken together (including the ground-based measurements of Zyskin and Mukanov¹⁰), in particular that the already very high $\dot{P}$ ($>2 \times 10^{-9}$ ss⁻¹) between 1972 and 1979 seems to become higher still between 1979 and 1983.

Finally, the 'spindown' time of this object hints at the possibility that Geminga may be the remnant of the event witnessed in AD 437 by the Chinese, which was almost certainly not a supernova, but simply a star "seen by day".

A simple phenomenological model of the source is proposed, which considers a binary system that includes a dwarf non-degenerate star and a collapsed object.

The X-ray data

Table 1 lists the X-ray observations of Geminga. Five data sets are given for the 1979–83 period: two Einstein imaging proportion counter (IPC) fields (3333 and 10371), one Einstein High Resolution Imager (HRI) field (8353) and two contemporary Exosat channel multiplier arrays (CMA) fields. The source data on each observation were analysed separately for periodicity in the ~ 59 -s range. Starting with the IPC 10371 data, where the best statistics are available, we now describe the analysis procedure. It consists of two steps: the source photon definition and the periodicity search itself. The IPC data suggest that Geminga is a very soft source (see ref. 6), most of the counts of which are within the first 10 of the 32 IPC energy channels. Considering also the shape of the IPC background spectrum,

Table 2 shows the signal and noise contributions to the observed source counts for three energy intervals of the IPC range; obviously, the best source signal-to-noise ratio is expected for energy channels 2–10. The relevant source photons are then defined by means of a saturation curve of the point spread function (PSF) of (in this case) the IPC instrument (Fig. 1). The number of total photons within a given radius R (in pixels of 8 arc s) from the source position is plotted against the radius R , together with the number of background photons expected. The number of background photons can be accurately determined from studying adjacent source-free regions and is remarkably constant. One can then subtract the background contribution from the total source counts, and obtain a 'pure source' curve (an integral PSF) which saturates (flattens) for $R = 23$ –25, or at a slightly greater value than the usual one for harder sources of similar flux (see ref. 11). One can thus fix, *a priori*, $R \leq 23$ and $2 < E_{\text{ch}} < 10$ as the selection criteria yielding the best signal-to-noise ratio for this source. This gives 810 photons, the arrival times of which were subjected to a periodicity search: they are folded in phase with a period scanning the region around the extrapolation of the $P(t)$ law joining the SAS 2 and COS B points: 59.40 to 59.80 s. This was covered in 20 steps of 2×10^{-2} s. Such steps are reasonably independent: over the elapsed $\approx 20,000$ s of observation, there are ~ 300 ~ 1 -min periods, and for a 6-s bin (in a 10-bin light-curve), it takes a period

Table 1 Observations

	Date	Elapsed time (s)	Useful time (s)
Einstein			
IPC 3333	20 September 1979	35,676	2,401
IPC 10371	17 March 1981	19,702	8,361
HRI 8353	18 March 1981	8,397	2,411
Exosat			
CMA 1	9 September 1983	30,676	19,106*
CMA 2	9 September 1983	34,263	27,496*

* Continuous observations; the difference between elapsed and useful times is due to instrumental dead time only.

Table 2 Energy channels

	2–32	2–20	2–10
Total counts	1,129	1,023	810
BKG counts	220	140	89

change of 0.02 s for a 1-bin shift. A maximum reduced χ^2 of 3.94 (9 d.f.) was found for $P = 59.74$. A further fine scan was carried out around this value, yielding a χ^2 of 4.26 (9 d.f.) for $P = 59.737$ s. This has a single trial probability of 1×10^{-6} , and considering the number of trials, a final probability of 2×10^{-5} . As a further check, a set of 'calibrations' was carried out, covering the region 10–210 s, where the data behaved as expected from classical statistics, indicating that the data base is not polluted by systematic time effects of unknown origin. Figure 2a displays the light curve corresponding to $P = 59.737$ s. When the background level (of ~ 100 photons) is subtracted, it is clear that a significant ($\sim 50\%$) 'unpulsed' component is also present.

Owing to the limited time interval covered by the Einstein observations (<3 h), no assumption was necessary about the existence of a $\dot{P}$, nor was there any scan for $\dot{P}$. On the other hand, it is possible to perform other tests to check the reality of the effect, and in particular, its behaviour according to the signal-to-noise ratio. After the maximum χ^2 was found, the corresponding period was used to fold the source data for different R values (in pixel, see Fig. 1). The right-hand scale of Fig. 1 gives the χ^2 values for that period ($= 59.737$ s) as a function of R . As expected, this goes through a maximum, peaking broadly around $R \sim 23$, where the 'pure source' counts cease to increase, and then decreases for greater R s, as mostly background photons are being added.

A further test, also *a posteriori*, is to introduce in the data base those photons with energy channel assignment >10 , that is, those that are mostly background. The resulting 1,190 photons, folded at the best period (59.737 s), yield a $\chi^2 = 3.8$, that is, degraded by the background introduction. We thus conclude, from the numerical value of the probability as well as from the signal behaviour with respect to noise perturbation, that the periodicity effect in the IPC 10371 photons from this source is significant. We also note that the general shape of the light curve appears to be regular, with two well defined peaks separated by ~ 0.4 – 0.5 phase.

The Einstein HRI observation 8353 was performed <12 h after IPC 10371, on 18 March 1981. Such a small lapse in time means that the $\dot{P}$ of the source can again be neglected and the HRI data can be analysed using the same value for the period. The source is seen very well in the HRI, with 91 counts within 15 pixels (of 0.5 arc s), and the counter background in this case is extremely low. The folding algorithm was then applied to the HRI data, yielding the 'light curve' shown in Fig. 2b, for $P = 59.737$ s and assuming the same time origin (phase) as for the IPC photons of Fig. 2a. Within the very limited statistics, the shapes of Fig. 2a and b are the same. Because of the common time origin, the IPC and HRI photons can then be folded together, producing the light curve in Fig. 2c where the background level (of 100 photons) is indicated. The reduced χ^2 is now $= 4.72$, or a single trial probability of $\sim 5 \times 10^{-7}$ and (considering that no scans were performed for the HRI data), a final confidence level of $\sim 1 \times 10^{-5}$ for the periodicity of 59.737 s, in the Einstein March 1981 observations. Figure 3 shows the χ^2 distribution for the *a posteriori* period scan for the complete IPC + HRI data set. The scan interval from 59.4 to 60.0 is shown for both the source data and the background. The source data show the reported periodicity as an apparent peak in the χ^2 distribution, while no such effect is seen in the off-source data. Note that, owing to the increased time base covering the IPC and HRI observations, the length of an independent period step is $\sim 7 \times 10^{-3}$ s. Figure 3 can also be used to assess the error of the best period determination. Within the limited statistics, the size of the peak around 59.73 s indicates an uncertainty of ± 0.05 s.

The same analysis procedure as for IPC 10371 was then applied to the IPC 3333 data. Here, the source statistics are poorer, owing to the shorter ($\approx 3,000$ s) exposure of the field. The source is, nevertheless, clearly visible, and is similar to the 10371 observation, in terms of both the saturation curve (which follows Fig. 1) and spectral shape. Here, the period expected

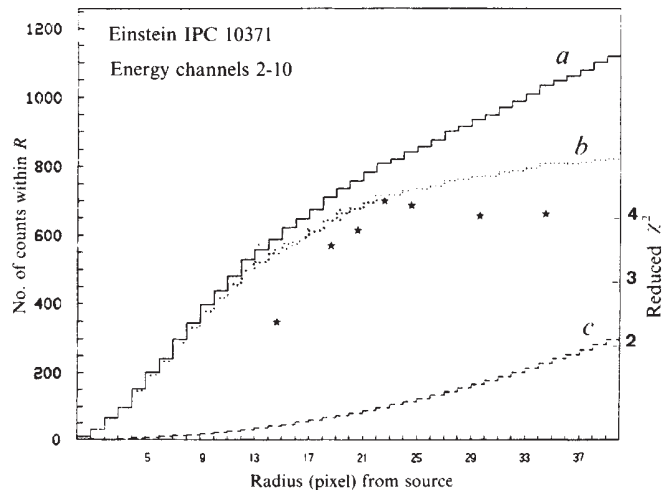


Fig. 1 Saturation curve of the Geminga source from the Einstein Observatory IPC 10371 observation. Left-hand scale: the number of photons in the 2–10 IPC energy channels within a given R (in 8 arc s pixels) plotted against R for: *a*, total; *b*, pure source or, total minus background; *c*, background, as measured for several adjacent source-free regions and assumed smooth. While the background contribution increases quadratically with R , the source is seen to 'saturate' for $R \geq 23$. Right-hand scale: reduced χ^2 value of the 10-bin light curve after folding the data at $P = 59.737$ s for different R values.

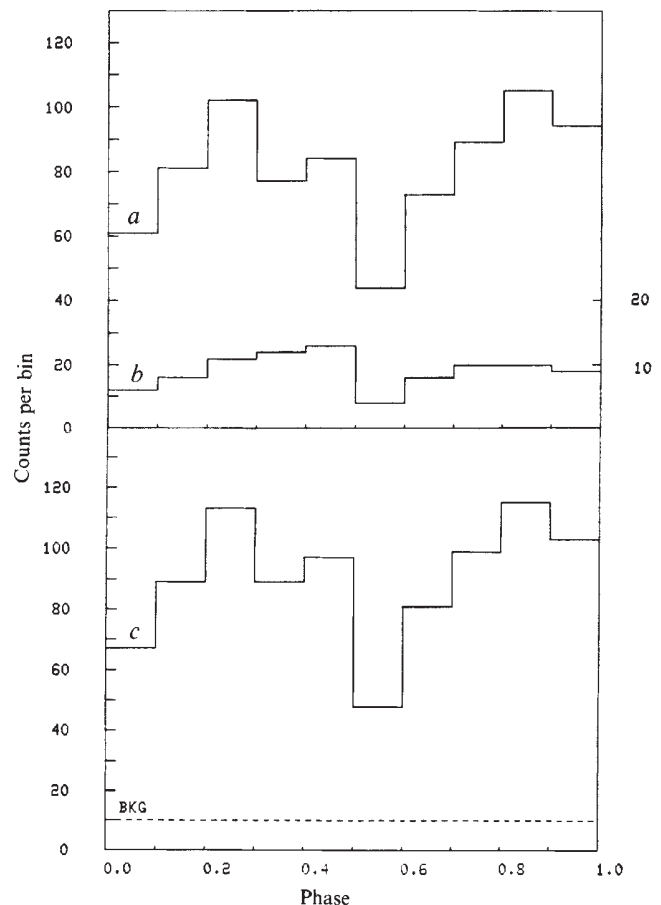


Fig. 2 Light curves for the Einstein March 1981 observations. *a*, IPC data only (energy channels 2–10, $R \leq 23$), 810 events folded at $P = 59.737$; *b*, HRI data only (91 events) (right-hand scale) folded at the same period and in phase with *a*; *c*, sum of the IPC and HRI light curves (911 events), the reduced χ^2 (9 d.f.) is 4.72. The background level is also shown.

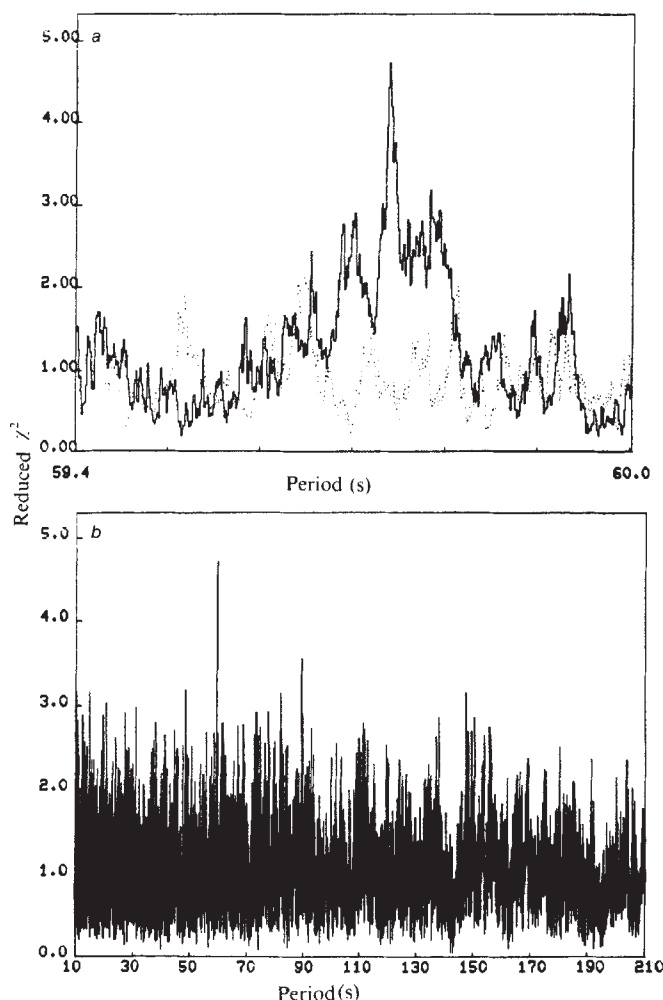


Fig. 3 Examples of the *a posteriori* period scans for the IPC 10371 + HRI 8353 Einstein source data. *a*, Total calibration scan, covering the period range 10–210 s in 10,000 steps of 0.02 s each. The data are seen to behave as expected from statistics, and the χ^2 peak (4.72) at ~ 59.7 is apparent. *b*, 'Zoom' of the above scan, covering the period interval 59.5–60.0 s in 600 steps of 0.01 s each; solid line: 901 source events; broken line: 1,295 background (off-source) events. The significance of the periodicity in the source data can be gauged by shape and width of the χ^2 peak between 59.7 and 59.8 s.

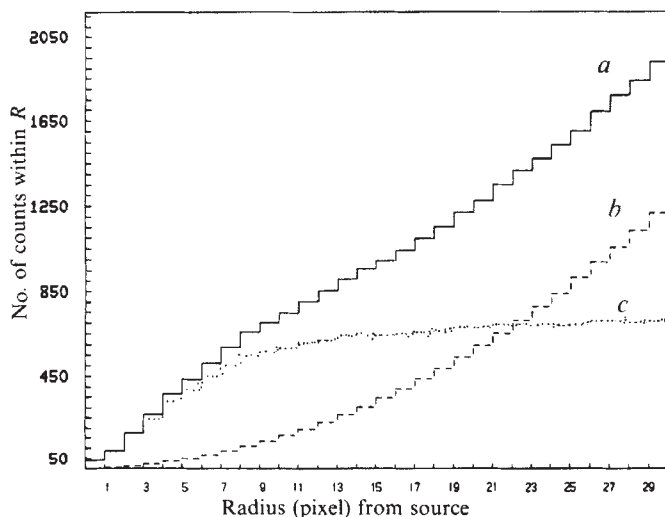


Fig. 4 Saturation curve of Geminig in the Exosat CMA2 observation (September 1983). Thin Lexan (3,000 Å) filter was used. Each pixel is 4 arc s. Curves *a*, *b* and *c* are as used in Fig. 1.

from the extrapolation of the SAS 2 and COS B data falls in the range 59.30–59.70 s. A similar timing analysis has been performed in this region on the 208 photons with $R \leq 203$ and energy channel 2–10. A maximum χ^2 of 3.6 (9 d.f.) was found for $P = 59.466$ s, corresponding to a single trial probability of 2×10^{-4} , reduced to 4×10^{-3} when the number of independent trials are taken into account.

The *a posteriori* checks on the signal-to-noise behaviour of the χ^2 values were also performed, and yielded results compatible with those obtained for IPC 10371. We conclude that, although not fully significant, the analysis of the IPC 3333 data suggests that the same effect is present, albeit at a period $P = 59.466$ s, implying a very strong period derivative (in excess of $4 \times 10^{-9} \text{ s s}^{-1}$) between the two Einstein observations. Note that from the IPC 10371 data one expects a 50% modulation, or, in the case of IPC 3333 observation, an effect due only to ~ 100 IPC source photons.

We now consider the more recent X-ray observations of this source, performed by Exosat in September 1983. The instruments used were the CMAs of the two (separate) low-energy telescopes, pointed in parallel at the source for ~ 10 h. The source detection and its possible time variability on a time scale of hours have been reported by Caraveo *et al.* (see accompanying report)⁷. Concentrating on the CMA2 data, obtained with a thin filter (3,000 Å Lexan), transparent to this soft source, it is possible to produce (Fig. 4) a saturation curve similar to those obtained for the Einstein Observatory IPC data, with each pixel here being 4 arc s. The background level (also checked to be uniform in adjacent source-free regions) is much higher for these data than for the Einstein ones, possibly also due to the very different orbital characteristics. Here again, the source is seen to 'saturate' later than expected from the counter performances (see refs. 7, 12) certainly due to the >20 arc min distance of this source from the telescope pointing direction. Thus, to have a result comparable with those of Einstein, rather than using a large R value (which would obviously accept a lot of background), we use an R value which implies the same signal-to-noise value as in the IPC data, that is, 10 when $R = 5$. The folding algorithm was then applied to the 360 events of the CMA2 with $R < 5$. The period scan region was, of course, influenced by the Einstein results, and covered the range 60.0–60.3 s, in steps of 0.01 s, or the value defining an independent step for a ~ 10 h observation of this periodicity. A maximum χ^2 of 3.09 (9 d.f.) was obtained for $P = 60.06$. A further small fine scan was carried out around this value yielding $\chi^2 = 3.24$ for $P = 60.056$; Fig. 5a shows the corresponding light curve.

To exploit all the available Exosat data, the photons detected at the same time in the CMA1 instrument, equipped with an aluminium/parylene (Al/Par) filter were used. Figure 5b shows the 'light curve' of these 75 photons, also folded in phase with the CMA2 photons at $P = 60.056$ and with $R \leq 5$. Although the CMA1 data are clearly non-significant the similarity of features in the two independent distributions of the two separate CMA1 and CMA2 instruments is interesting. Moreover, the sum of the two curves, that is the total CMA data or 435 photons, shows a significant χ^2 improvement ($=3.86$), and is also shown in Fig. 5c. The overall probability of this light curve being due to a random fluctuation is $\sim 1 \times 10^{-3}$.

The same *a posteriori* calibration procedure as used for the Einstein data was applied to the Exosat CMA data: the source data were folded over the period 10–210 s. While the data exhibited the expected statistical behaviour, no other significant periodicity was found in the range 59–60 s.

No absolute phasing has yet been performed between Figs 2 and 5. This, as well as the different statistics and instrument types, renders the comparison of the IPC and CMA light curves difficult. However, some similarities in shape can be seen (for example two peaks) and the CMA light curve again implies a $\sim 50\%$ source modulation. If anything, differences in the peak shapes could be attributable to the different energy ranges covered by the various counters and/or to the different epochs of observations.

The Exosat data, taken individually, provide a good independent confirmation of the existence of the periodicity effect in Geminga, with an overall confidence level between those of the two Einstein data sets, as can be predicted from the statistics and the counter performances.

As a general check against possible systematic origin of both the Einstein and Exosat periodicity results, a calibration procedure (consisting of 50,000 steps between 10 and 110 s) was applied for off-source data, taking a comparable number of photons for each case. In no case did the reported periods have an even remotely significant value of χ^2 , thus ruling out possible instrumental causes for such periodicities in the data from the two satellites. The dotted curve of Fig. 3 gives an example of the results of the folding on off-source data.

The γ -ray data

The available observations in γ rays published so far come from the SAS 2 and COS B missions in the range >50 MeV, in refs 2 and 8 and the two ground-based ones $>10^{12}$ eV, in ref. 10. It is not easy to assess the confidence levels individually, and even less so to compare them, beyond the evaluations given in each paper. While the two ground-based measurements were relatively short (1–2 days), for the two satellite missions, due to the observation lengths, a $\dot{P}$ term had to be introduced, leading to a bidimensional P – $\dot{P}$ scan in the periodicity search, which increased the number of trials. However, the number of trials considered in the COS B retraction of the effect⁹ was probably not correct, owing to the obvious non-independence of the trials in the P and $\dot{P}$ scans. While awaiting further analysis of the COS B data, we take the published P and $\dot{P}$ at their face values.

The complete secular picture

Having reviewed all the available data on the 59-s periodicity of Geminga, that is, two different satellite γ -ray (>50 MeV) observations, two ground-based $>10^{12}$ eV γ -ray measurements and five different X-ray satellite (few keV) data sets, we can present the secular picture, covering the period December 1972–September 1983. This is given in Fig. 6, where the period values are plotted against time. The vertical error bars of the X-ray data are derived using the method illustrated in Fig. 3. Even for the SAS 2 and COS B data, the duration of the measurement is unnoticeable on the graphic scale. For clarity, the earlier reported errors on the $\dot{P}$ (refs 2, 8) are not plotted.

All the points shown in Fig. 6 have a common secular trend, except for the 1981 measurements of ref. 10, which has a period of 59.28 s. Although the number of γ -rays used for this point is exactly half of those for the 1979 point at 59.46 s, the authors claim a higher level of confidence for it. We shall propose below a possible explanation for the position of this point; but first, we examine the secular trend of the other six measurements.

Three possibilities of fitting the data with a $P(t)$ law were investigated: (1) a straight line; (2) a second-order polynomial; and (3) two straight lines. The first gives a very poor fit and will be disregarded. Good results could be obtained with a single parabola, while with two straight lines several possibilities exist, all imposing a significant break in the slope somewhere between 1979 and 1980. The best fit, in this last assumption, is obtained using $\dot{P} = 2.4 \times 10^{-9}$ up to Modified Julian Day (MJD) $\geq 11,000$ and $\dot{P} = 4.68 \times 10^{-9}$ thereafter (Fig. 6).

Assuming that the reported periodicity is due to a compact body rotation, and its rate of change to a secular slowing down of this rotation, the fits of Fig. 6 can be used to evaluate its age. In the parabolic fit, the continuously changing $\dot{P}$ reached 0 about 15 yr ago, an interesting if extreme suggestion. In the linear fit, it is uncertain which value of P should be used for computing the spindown time $\tau = P/\dot{P}$ in view of the suggested change, which, if true, can hardly be considered a unique event in the object life. For the 1973–79 $\dot{P}$, which seems to be very well represented by a single straight line, the spindown time would be ~ 800 yr; this time would have to significantly

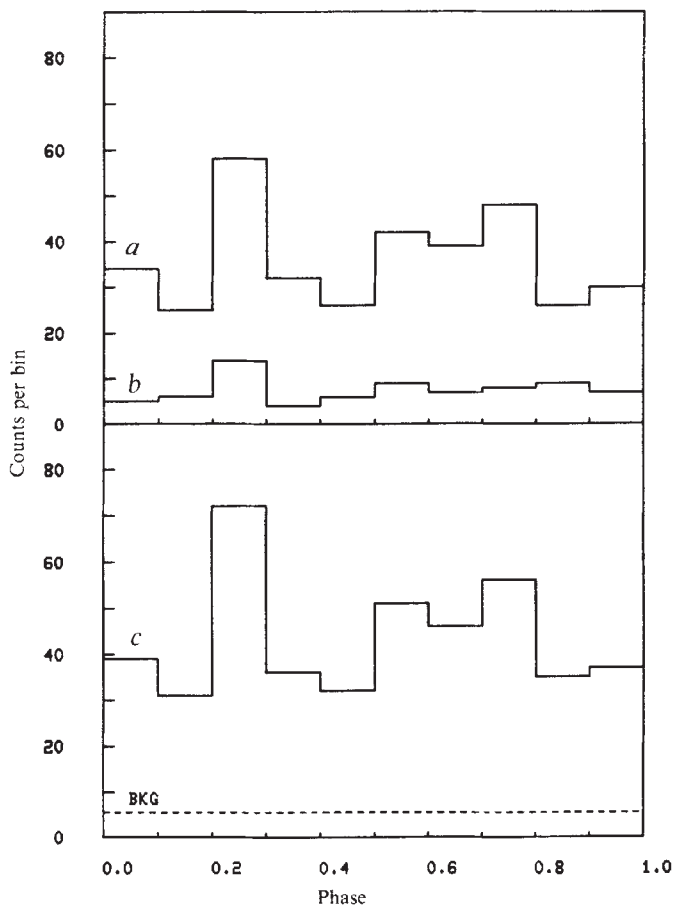


Fig. 5 Light curves of the Geminga Exosat CMA data folded in phase with $P = 60.056$ s. *a*, CMA2 (Lexan) data (360 events). *b*, CMA1 (Al/Par Filter) data (75 events). *c*, Sum of the *a* and *b* data, yielding a reduced $\chi^2 = 3.86$ (9 d.o.f.). The background levels are also shown.

lengthened if similar changes in P had occurred earlier in the object's lifetime. On the other hand, if similar changes in $\dot{P}$ occur frequently enough (perhaps once every 10 yr) then a very small value of $\dot{P}$ can be reached in less than a few hundred years.

Note that there is a preliminary report (P. Dourouchoux and A. S. Jacobson, personal communication) of an intense source of 1 MeV γ rays seen from the general Geminga direction during one HEAO 3 observation early in 1980, but not during the preceding one in 1979.

The event of AD 437

When studying Geminga, it is interesting to recall the event recorded by the Chinese in AD 437 (ref. 10) when, on 26 January, a star was "seen by day" (actually between 3 and 5 p.m.) in the Lunar Mansion "Tung Ching"¹⁵. Stephenson¹³ translates this into galactic coordinates $l = 195$, $b = +5$, a rather striking coincidence with 2CG195+4. The uncertainties on these coordinates, however, are unknown and may be large (F. R. Stephenson, personal communication). From the considerations on $\dot{P}$ and its possible changes, the 'age' of the object, or at least its spindown time, could be compatible with 1,500 yr, even if this value is not implied by the data. On the other hand, the event of AD 437 is still not identified, because early attempts to associate it with the SNR IC443 have now been shown to be untenable. The analysis by Stephenson and Clark¹⁴ suggests that the event was not due to a supernova, mostly because of its short duration. Moreover, no remnant of a supernova explosion of 1,500 yr ago (at least of the known types and especially if at a distance of ≥ 100 pc or so) could have remained undetected

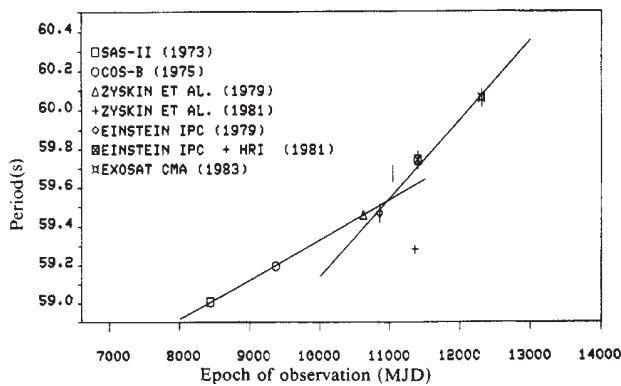


Fig. 6 Secular variation of the 59-s periodicity of Geminga. Over the 1973–83 decade, the period values for each observations (see symbols where the measurement year is specified) are plotted against MJD. As an example of the good fit to the data, two lines are shown with slopes $\dot{P} = 2.4 \times 10^{-9} \text{ s s}^{-1}$ up to MJD $\leq 11,000$ and $\dot{P} = 4.68 \times 10^{-9}$ thereafter. The time of the positive HEAO 3 ~ 1 MeV γ -ray measurement is shown with a vertical bar at MJD 11040.

in the Geminga region. One is then left to consider an event producing a relatively short optical flash, reaching an apparent visual magnitude of ~ -4 (ref. 15), rather brighter than an ordinary nova.

Constraints on the model

The evidence summarized in Fig. 5 suggests a source with the highly unusual property of an emission covering the keV–TeV range with a periodicity around 1 min and which, moreover, shows a high and rapidly increasing period derivative. This is not an easy problem to interpret.

Maraschi and Treves¹⁶ noted, at the time of the first COS B observation, that an explanation for Geminga requires a choice between an isolated compact object (neutron star), or one in a binary system. For the isolated neutron star, a difficulty with the energy budget arises if the observed P and $\dot{P}$ are interpreted as being due to the spin down of a pulsar. Following Maraschi and Treves, the available energy loss $\dot{E} = I\omega\dot{\omega}$, where ω is the rotational angular velocity and I is the moment of inertia, would be in the region of 10^{32} – 10^{33} erg s, and even if this were transformed with a high efficiency in γ ray emission (both ~ 100 MeV and $> 10^{12}$ eV assuming that these could be produced by such a slow pulsar), it would require a distance to the source of several tens of parsecs only. While this is not in contrast with the X-ray source distance estimated from interstellar absorption, there is no independent evidence for such a small distance. Maraschi and Treves proposed that the observed ~ 1 -min period is that of the neutron star nutation, and that the true spin period, much shorter, is so far undetected. This *Deus ex machina* would supply, as in the case of Cyg X-3, all the energy needed, and one would only be concerned with satisfying the observational constraints on the flux at various wavelengths. We have scanned the data using a Fourier fast transform algorithm for unknown short periodicities, but without success. Admittedly, this is a difficult search, especially in the presence of a strong and variable $\dot{P}$. Note that, again following Maraschi and Treves, if the ~ 59 -s period P_p is interpreted as due to the precession of an object spinning with period P_s , then $P_p/P_s = \dot{P}_p/\dot{P}_s$, implying a $\dot{P}_p$ comparable with that of, for example, the Crab pulsar for a similar P_s . One would thus face the opposite situation—much more power than is observed—and would have to invoke a very unfavourable beaming geometry.

The alternative possibility, that of a binary system, appears more attractive because of the added degree of flexibility and also because of its better fit to the optical observations gathered

so far^{5,17,18}. The $m_R \geq 20$ candidate could be a subliminous G-type star in a binary system with a collapsed object (which in itself could hardly yield the observed $B - V \sim +0.6$). This hypothesis could explain the 1981 point of Zyskin and Mukanov at 59.28 s. As, for example, in the proposal by Vestrand and Eichler¹⁹ for Cyg X-3, a beam of ultrahigh-energy particles is created in the vicinity of the neutron star and hits the surface of the primary. Radiative collisions (π^0 and bremsstrahlung production) reprocess the incident beam to produce the observed GeV–TeV γ rays and the (possibly thermal) keV X rays. For a given angular width of the primary beam, and if the reprocessing introduces some further scattering, the possibility exists for the reprocessed radiation to be observed together with the 'direct' ultrahigh-energy beam. Co-rotation of the processing region would produce a beat period between the reprocessed emission and the direct one, the difference in frequency between the two being the orbital frequency. At variance with the quoted Cyg X-3 model, this is similar to what is observed, at different wavelengths, in some X-ray binaries and cataclysmic variables (see ref. 20).

Assuming then that the 1981 ultrahigh-energy point is at the 'direct' period of 59.28 s, from Fig. 6 one would place its appropriate beat at 59.65 s, and thus compute the orbital period of the system, which turns out to be 160 min.

The immediate difficulty would then concern the other (1979) point of ref. 10, which, at 59.46 s, seems to be well aligned with the $\dot{P}$ fit of Fig. 6. If 10^{12} eV γ -rays are also created by radiative collision reprocessing, this could be interpreted as a beat period itself, and imply that two period values, separated by an orbital frequency of 1×10^{-4} Hz, could be present in the ultrahigh-energy γ -ray data with possible different statistical significance and, of course, of difficult detection within limited statistics.

In the above picture, the energy budget is not particularly eased with respect to the isolated neutron star situation, the main advantage being that, if precession is invoked to explain the 59-s period, the reprocessing geometry can help to eliminate the excess power generated by a fast pulsar.

Many questions remain unanswered, especially if the observed periodicity is not due to precession. What could be the reason for such a high positive $\dot{P}$? Could it be connected with angular momentum transfer in a binary system, remembering that accretion must be limited to yield an X-ray luminosity of $\sim 10^{30}$ erg s⁻¹ (at 100 pc)? What is the cause of its apparent (and lasting) change? Could this be connected^{21,22} to the 'transient' low-energy γ -ray event at MJD 11040 (early 1980)? Is the obviously short lifetime of the system related to an evolutionary stage of a longer (binary) scenario? What about its origin? If the correlation with the AD 437 Chinese observation holds (in a way, this seems easier to accept than more radically short lifetimes) and because no SNR is observed with any certainty, a non-explosive collapse should be considered (as mentioned by Maraschi and Treves¹⁶), for example, with the mechanism proposed in ref. 23.

Conclusions

We have presented evidence for periodicity at ≥ 59 s for five independent X-ray data sets of the Einstein and Exosat source within the Geminga γ -ray error box. This also confirms the early discovery in γ rays (SAS 2, COS B) of such an effect, and, more importantly, gives final proof of the identity of the X- and γ -ray sources. For the X-ray data, the evidence is quite firm on the existence of a significant ($\approx 50\%$) unmodulated flux from the source. The complete data on this source, including satellite X- and γ -ray data and ground-based ultrahigh-energy γ -ray data, point to a very strong positive $\dot{P}$ for such a pulsation, which seems to exhibit either a continuous secular change in the years of observations (1973–83) or a sharp increase of about a factor of 2 in 1980.

Some corollary points have also been made. The 'age' ($= P/\dot{P}$) of the 'pulsating' object, as well as the coordinates in the sky, could associate its origin with the event witnessed by the Chinese

in AD 437. In view of independent evidence on the proposed optical counterpart, a qualitative model can be constructed, based on a compact object (for example, neutron star) in a binary system. In this model, reprocessing by radiative collisions of an ultrahigh-energy beam would explain most of the keV–TeV data, which would be seen at a beat of a true rotational period of the compact object. A binary period of ~ 160 min would be implied. The 'transient' HEAO 3 γ -ray flux of 1 MeV suggested to originate from the Geminga direction could be correlated to the time (early in 1980) of the mentioned $\dot{P}$ increase.

The data accumulated over a decade of Geminga observations and, in particular, the X-ray observations, have yielded sig-

nificant progress towards our understanding of this object. It is too early to say whether the rest of the COS B unidentified γ -ray objects have anything in common with this particular source, which was discovered through the γ -ray channel but would never have been seen if it were only at ≥ 4 times the distance.

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1. Fichtel, C. E. *et al. Astrophys. J.* **198**, 163 (1975).
2. Thompson, D. J., Fichtel, C. E., Hartman, R. C., Kniffen, D. A. & Lamb, R. C. *Astrophys. J.* **213**, 252–262 (1977).
3. Swanenburg, B. N. *et al. Astrophys. J. Lett.* **243**, L69–L73 (1981).
4. Bignami, G. F. & Hermsen, W. *Rev. Astr. Astrophys.* **21**, 67 (1983).
5. Caraveo, P. A., Bignami, G. F., Vigroux, L. & Paul, J. A. *Astrophys. J. Lett.* **276**, L45–L47 (1984).
6. Bignami, G. F., Caraveo, P. A. & Lamb, R. C. *Astrophys. J. Lett.* **272**, L9–L13 (1983).
7. Caraveo, P. A., Bignami, G. F., Giommi, P., Mereghetti, S. & Paul, J. A. *Nature* **310**, 481–483 (1984).
8. Masnou, J. L. *et al. Proc. 12th ESLAB Symp.* 33 (ESA SP 124, 1977).
9. Masnou, J. L. *et al. 17th I.C.R.C. Paris* 1, 177 (1981).
10. Zyskin, Yu. L. & Mukanov, D. B. *Soviet. Astr. Lett.* **9**, 117–118 (1983).

11. Hertz, P. L. & Grindlay, J. E. *Astrophys. J.* (in the press).
12. *Exosat Observer Guide* ESA (1984).
13. Stephenson, F. R. Q. *Jl R. astr. Soc.* **17**, 121–124 (1976).
14. Stephenson, F. R. & Clark, D. H. *Monogr. astr. Subjects* **4** (Hilger, Bristol, 1978).
15. Xi Ze-Zong & Po Shu-jen *Science* **155**, 597–603 (1966).
16. Maraschi, L. & Treves, A. *Astr. Astrophys.* **61**, L11–L13 (1977).
17. Halpern, J. P., Grindley, J. E., Bignami, G. F. & Caraveo, P. A. *IAU Circ. No.* 3907 (1984).
18. Halpern, J. P. & Grindley, J. E. *Bull. Am. astr. Soc.* **15**, 909 (1983).
19. Vestrand, W. T. & Eichler, D. *Astrophys. J.* **261**, 251 (1982).
20. Warner, B. in *Cataclysmic Variables and Related Objects* (eds Livio, M. & Shaviv, G.) 155 (Reidel, Dordrecht, 1983).
21. Katz, J. I. in *Positron–Electron Pairs in Astrophysics* (eds Burns, M. L., Harding, A. K. & Ramaty, R.) 65 (American Inst. of Physics, 1983).
22. Greenstein, G. *Astrophys. J.* **231**, 880–895 (1979).
23. Canal, R. & Schatzmann, E. *Astr. Astrophys.* **46**, 229–235 (1976).

Malignant transformation of early passage rodent cells by a single mutated human oncogene

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When linked to transcriptional enhancers, the mutant Ha-ras-1 gene from the T24 bladder carcinoma cell line induces the complete malignant transformation of early passage cells, while the normal Ha-ras-1 proto-oncogene only induces immortalization. Therefore, mutated Ha-ras-1 does not require a cooperating gene to trigger malignant conversion and ras genes may be involved in the process of tumorigenesis at an earlier stage than previously suspected.

CARCINOGENESIS *in vivo* has been shown to be a multi-step process. Evidence for this comes from epidemiological studies¹, experimental chemical carcinogenesis in animal model systems² and recent studies on virally induced tumours in chickens^{3,4}. The process has broadly been subdivided into two steps, an initiating step and a completing step⁵. Initiation is thought to be an absolute requirement involving mutational events in as yet unknown genes, while completion involves subsequent alterations that result in the tumorigenic state. In attempts to develop experimental systems suitable for *in vitro* testing of this model, much recent work has focused on the tumorigenic conversion of cells in culture^{6–8}. In such systems two steps can again be distinguished: a first step in which cells are immortalized (rescue from senescence) but have few phenotypic characteristics of malignant cells, and a second step (completing step) in which cells acquire phenotypes characteristic of malignant cells (for example, reduced serum requirement, anchorage independence and ability to induce tumours in experimental animals).

Initial attempts to identify genes acting dominantly at the cellular level, which could affect one of these steps, concentrated on viral genes. As a result of these experiments several viral genes have been identified which are thought to effect rescue from senescence but not completion. These include the polyoma

large T antigen and the adenovirus Ela genes^{9,10}. Genes which exhibit the ability to cause completion to a malignant phenotype include the adenovirus E1b and polyoma virus middle T genes^{11,12}. Another set of viral genes (termed *v-onc* genes) involved in the malignant conversion of cells has been identified in the acutely transforming, highly oncogenic retroviruses. Few of these have been rigorously tested in quantitative assays for rescue from senescence or completion, but it is clear that at least some cases, retroviral oncogenes can trigger complete malignant conversion of primary cells (for reviews see refs 13, 14). In these cases, it seems likely that both mutation of amino acid coding sequences and transcriptional activation contribute to the highly transforming phenotype. The retroviral oncogenes are closely related to and are assumed to be derived from normal cellular genes (termed proto-oncogenes). Alterations in these genes are associated with *in vivo* carcinogenesis. The alterations so far identified include amplification, rearrangements such as transposition or chromosomal translocation and mutation (for review see ref. 15).

DNA-mediated gene transfer techniques are among the most useful ways of detecting altered cellular proto-oncogenes with transforming ability in tumour cells and cell lines^{6–8}. Most of these experiments have used NIH 3T3 cells as recipients, and most experiments have detected mutated cellular homologues

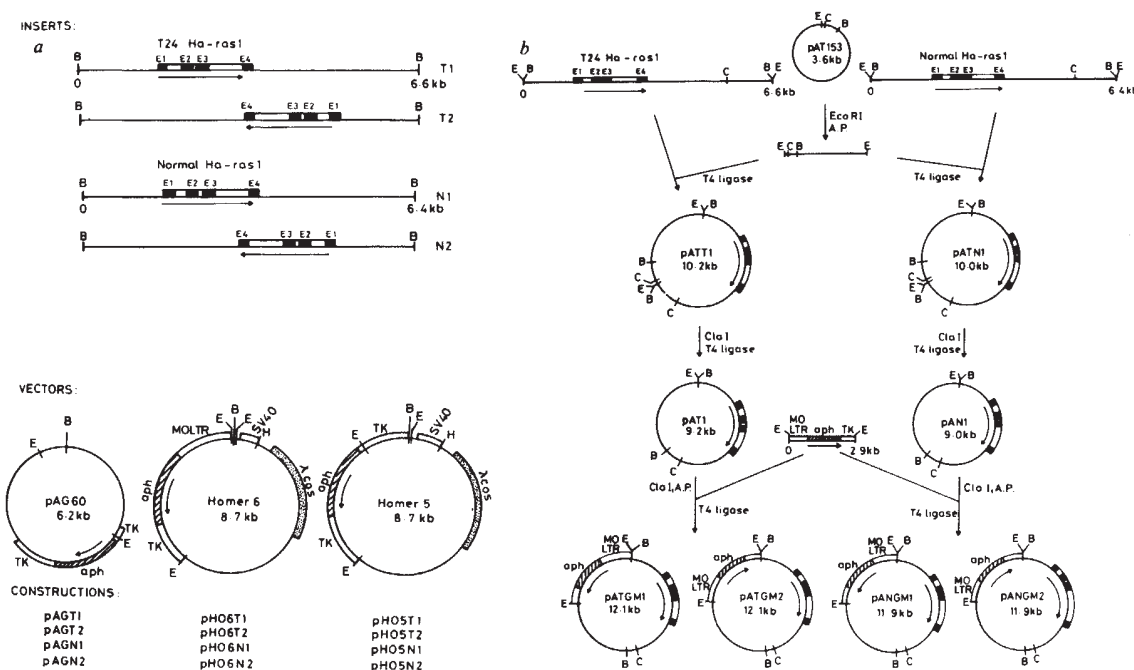


Fig. 1 **a**, Schematic representation of pAG60 (6.2 kb), Homer 6 (8.7 kb), Homer 5 (8.7 kb) and derivative plasmids carrying the human T24 or normal Ha-ras-1 gene. The *Bam*HI fragment containing the T24 Ha-ras-1 oncogene is 6.6 kb long²², whereas the *Bam*HI fragment containing the normal human Ha-ras-1 oncogene from fetal liver is 6.4 kb³⁰. Plasmid pAG60³¹ contains the bacterial Tn5-encoded aminoglycoside phosphotransferase (*aph*) gene under the transcriptional control of the 5' and 3' signals of the herpes simplex virus thymidine kinase gene (HSV-1 *tk*). Plasmids pAGT1 and pAGT2 were obtained by insertion of a 6.6-kb *Bam*HI fragment containing the human T24 bladder carcinoma Ha-ras-1 oncogene into the *Bam*HI site of pAG60, and plasmids pAGN1 and pAGN2 by insertion of the 6.4-kb *Bam*HI fragment containing the human normal Ha-ras-1 oncogene. Plasmids pAGT1 and pAGN1 contain the T24 and the normal Ha-ras-1 genes in the same orientation as the *aph* gene and plasmids pAGT2 and pAGN2 in the opposite orientation. Homer 6 was derived from the cosmid vector Homer 5 by Jonathan Wolf. Homer 6 contains the *aph* gene under the 5' transcriptional control of the Moloney murine sarcoma virus long terminal repeat (LTR) promoter and enhancer regions³² and the 3' polyadenylation signal derived from the HSV-1 *tk* gene. The MoMSV LTR-containing sequences consist of a 530-base pair (bp) *Eco*RI-*Xba*I fragment of cloned integrated MoMSV carrying 230 bp of mink sequences upstream of the remaining 300 bp of viral LTR sequences³³. The *Xba*I site in the MoMSV LTR was converted to *Bam*HI using molecular linkers. Homer 6 also contains the 430-bp *Hpa*II-*Hind*III fragment of SV40 spanning the origin of replication and carrying the 'enhancer' region and the packaging signal of phage λ (λ cos). Plasmids pHO6T1 and pHO6T2 were obtained by inserting the 6.6-kb T24 Ha-ras-1 oncogene fragment into the *Bam*HI site of Homer 6 and plasmids pHO6N1 and pHO6N2 by inserting the 6.4-kb normal Ha-ras-1 gene into the same site. Plasmids pHO6T2 and pHO6N2 contain the T24 and the normal Ha-ras-1 genes in the same orientation as the *aph* gene and plasmids pHO6T1 and pHO6N1 in the opposite orientation. Homer 5 differs from Homer 6 in that it contains the *aph* gene under the 5' transcriptional control of the HSV-1 *tk* gene instead of the MoMSV LTR. **b**, The construction of recombinant plasmids containing the normal or mutant Ha-ras-1 genes, the *aph* gene and a single MoMSV LTR enhancer. *Eco*RI fragments containing the T24 Ha-ras-1 gene (6.6 kb) or the normal Ha-ras-1 gene (6.4 kb) were cleaved from plasmids pHO6T1 or pHO6N1 (see **a**) and isolated from low melting agarose gels. The fragments were ligated to phosphatase *Eco*RI-cleaved pAT153 and plasmids pAT1 and pAN1 isolated. *Cla*I digestion followed by ligation removed a 1.0-kb fragment containing adjacent *Eco*RI and *Bam*HI sites producing plasmids pAT1 and pAN1 which contain single *Eco*RI sites. Ligation of the 2.9-kb *Eco*RI fragment containing the *aph* gene linked to the MoMSV LTR fragment (isolated from pHO6T1, see **a**) into the *Eco*RI sites of pAT1 and pAN1 produced plasmids pATGM1, pATGM2, pANGM1 and pANGM2. The maps are not drawn to scale; arrows represent the transcriptional orientation of the Ha-ras-1 and *aph* genes. AP, alkaline phosphatase; B, *Bam*HI; c, *Cla*I; E, *Eco*RI.

of the oncogenes contained in Harvey and Kirsten murine sarcoma viruses (*ras* genes)^{7,8}. As NIH 3T3 cells are considered to be an abnormal cell line, already rescued from senescence and predisposed to completion, it has been suggested that *ras* oncogenes are second-step transforming genes or 'progressogenes'^{16,17}. Several recent studies support this proposal¹⁸⁻²⁰. They suggest that the Ha-ras-1 oncogene from the human T24 bladder carcinoma cell line, which has a substitution at amino acid position 12, could cause the malignant conversion of early passage rodent cells only if they are immortalized by chemical carcinogens, or when a second gene mediating rescue from senescence is co-transferred.

Here we show that these requirements can be eliminated. The T24 oncogene alone can rescue low passage rodent cells from senescence and, if transferred to recipient cells in recombinant DNA constructions containing transcriptional enhancers, can also directly trigger low passage cells to convert to a fully tumorigenic phenotype. Moreover, the normal *ras* proto-oncogene can also rescue low passage rodent cells from senescence when introduced into recipient cells in high expression vectors.

Transformation

In order to introduce normal and mutant *ras* genes into low passage rodent cells, the cloned oncogenes were linked to a dominant selectable marker. The marker used was the aminoglycoside phosphotransferase gene (*aph*) from bacterial transposon Tn5, which confers on eukaryote cells resistance to the antibiotic geneticin (G418). *Bam*HI fragments containing either the activated or the normal Ha-ras-1 gene were introduced into the *Bam*HI site of three different bacterial plasmids containing the *aph* gene (Fig. 1). Plasmids pAG60 and Homer 5 contain an *aph* gene under the transcriptional control of the herpes simplex virus thymidine kinase gene (HSV *tk*) while in Homer 6 the 5' *tk* control sequences have been replaced by those of Moloney murine sarcoma virus (MoMSV) which contain a transcriptional enhancer. Both Homer 5 and Homer 6 include a fragment containing the simian virus 40 (SV40) origin of replication, which also contains an enhancer. Thus, in the pAG series, no known transcriptional enhancers are present, in the pHO5 series one enhancer (from SV40) is present, while in the pHO6 series two enhancers (from SV40 and MoMSV) flank the inserted

Table 1 Transfection of Chinese hamster and rat cells with *aph* recombinant plasmids

Recipient cells	Donor DNA	No. geneticin-resistant colonies per flask (liquid medium)		No. geneticin-resistant colonies per plate (semi-solid medium)	Recipient cells	Donor DNA	No. geneticin-resistant colonies per flask (liquid medium)		No. geneticin-resistant colonies per plate (semi-solid medium)
		Total No. av. $\pm$ s.d.	Morphologically transformed av. $\pm$ s.d. (%)				Total no. av. $\pm$ s.d.	Morphologically transformed av. $\pm$ s.d. (%)	
CHL(F3)	pAG60	3.3 $\pm$ 2.0	0	0	Rat embryo	pHO6T1	25 $\pm$ 5.2	23 $\pm$ 3.6 (9)	15 $\pm$ 2.9
	pAGT1	9.0 $\pm$ 4.3	0	0		pHO6T2	21 $\pm$ 4.9	20 $\pm$ 4.2 (95)	14 $\pm$ 5.9
	pAGN1	7.3 $\pm$ 3.8	0	0		pHO6N1	15 $\pm$ 4.5	0	0
	Homer 6	45 $\pm$ 12	0	0		Rat embryo	0	0	0
	pHO6T1	99 $\pm$ 13	92 $\pm$ 10 (95)	79 $\pm$ 17	Rat muscle†	pAG60	3.3 $\pm$ 2.1	0	0
	pHO6T2	89 $\pm$ 9.4	84 $\pm$ 8.3 (94)	71 $\pm$ 17		pAGT1	4.3 $\pm$ 3.0	0	0
	pHO6N1	80 $\pm$ 13	0	0		Homer 6	46 $\pm$ 8.3	0	0
	pHO6N2	83 $\pm$ 12	0	0		pHO6T1	95 $\pm$ 11	90 $\pm$ 6.7 (95)	76 $\pm$ 9.2
	Homer 5	38 $\pm$ 12	0	0		pHO6T2	54 $\pm$ 7.9	81 $\pm$ 7.5 (96)	54 $\pm$ 6.1
	pHO5T1	28 $\pm$ 7.3	25 $\pm$ 6.5 (89)	25 $\pm$ 6.8	Rat skin†	pHO6N1	69 $\pm$ 11	0	0
	pATGM1	73 $\pm$ 8.0	69 $\pm$ 7.0 (94)	65 $\pm$ 16		Rat embryo	0	0	0
	pATGM2	62 $\pm$ 17	59 $\pm$ 16 (95)	56 $\pm$ 16		pAG60	3.3 $\pm$ 2.2	0	0
	pANGM1	60 $\pm$ 12	0	0		pAGT1	3.8 $\pm$ 2.4	0	0
	pANGM2	67 $\pm$ 14	0	0		Homer 6	40 $\pm$ 15	0	0
	Salmon	0	0	0		pHO6T1	88 $\pm$ 7.0	83 $\pm$ 9.0 (94)	68 $\pm$ 13
Rat embryo†	pAG60	2.5 $\pm$ 1.3	0	0		pHO6T2	63 $\pm$ 14	59 $\pm$ 13 (94)	63 $\pm$ 20
	pAGT1	1.0 $\pm$ 0.8	0	0		pHO6N1	70 $\pm$ 9.3	0	0
	Homer 6	17 $\pm$ 4.7	0	0		Rat embryo	0	0	0

* Transfection of third-passage Chinese hamster lung cells CHL(F3) was carried out as follows: 100 ng of each superhelical plasmid DNA mixed with 10 μ g salmon sperm DNA as carrier was co-precipitated with calcium phosphate in a volume of 0.5 ml²¹ and added to 1×10^5 recipient cells in 5 ml medium per 25 cm² flask. 24 h later the medium was changed with 5 ml non-selective medium (SF12; Flow) containing 15% Hyclone serum (Sterile Systems Inc.) and incubation was continued at 37 °C for 24 h. The medium was then changed to selective medium containing 15% Hyclone serum and 200 μ g ml⁻¹ geneticin (Gibco). Duplicate 25-cm² flasks were trypsinized, the cells counted and 1×10^5 cells plated per 9-cm bacteriological plate in 20 ml Methocel medium (SF12 containing 0.9% Methocel (Fluka), 30% Hyclone serum and 200 μ g ml⁻¹ geneticin). The medium of liquid cultures was changed every 2–3 days for up to 10 days, when colonies were examined and counted using an inverted microscope. Foci consisting of predominantly highly refractile, pebble-shaped cells which contained occasional giant nuclei, and which grew in a disoriented manner were classified as morphologically transformed. Colonies were picked using cloning rings and trypsin after removing the top of the flask with a heated scalpel. Colonies in Methocel-containing plates were counted 10 days post-plating and picked with Pasteur pipettes. The data are derived from the results of four to six flasks or plates per donor DNA from two to three experiments.

† Transfection of second-passage rat (Wistar) cells was carried out as follows: 1 μ g of each superhelical plasmid DNA mixed with 10 μ g rat embryo DNA as carrier in 0.5 ml calcium phosphate DNA co-precipitate were added per 1×10^5 recipient cells (passage 2 at 1:4 split ratio) in 5 ml medium per 25-cm² flask. Selection of geneticin-resistant colonies in liquid or semi-solid medium was carried out as described above. The results are derived from four flasks per donor DNA.

fragments. We also constructed plasmids pANGM1, pANGM2, pATGM1 and pATGM2 containing the normal or the T24 Ha-ras-1 genes and the *aph* gene with a single MoMSV enhancer, by inserting a 2.9-kilobase (kb) *Eco*RI fragment containing the enhancer and the *aph* gene into plasmids pAT1 and pAN1 (Fig. 1b).

Recombinant DNAs were introduced into early passage rodent cells using the calcium phosphate precipitation technique first described by Graham and Van der Eb²¹. The ability of cells to take up and stably express the exogenous DNA was determined by measuring the number of geneticin-resistant colonies obtained with each cell type and donor DNA. The ability of donor DNA to rescue cells from senescence, regardless of morphology, was determined by isolating geneticin-resistant colonies and passaging the cells *in vitro*. In the growth conditions used here, individual hamster clones and Wistar rat cells senesced after approximately 30 doublings. (Cloned cells capable of *in vitro* passage for >100 doublings were considered rescued from senescence.) Second-step transformation (completion) was determined by the ability of the cells to grow in semi-solid media (anchorage independence).

Table 1 shows the results obtained on the introduction of 100 ng of recombinant DNA into passage 3 Chinese hamster lung cells (CHL(F3)). Geneticin-resistant colonies were obtained with all recombinant DNAs tested, but at different frequencies. As might be expected, plasmids containing known transcriptional enhancers produced more colonies than the pAG series which did not contain enhancers. Microscopic examination of the geneticin-resistant colonies revealed a most striking result. Colonies obtained after transfection with plasmids containing the normal Ha-ras-1 gene or the T24 Ha-ras-1 gene with no known enhancers appeared normal, with flat morphology, and exhibited contact inhibition. However, >90% of the colonies obtained with plasmids containing the T24 Ha-ras-1

gene and either or both the SV40 and MoMSV enhancers contained highly refractile, pebble-shaped cells which grew in a disoriented manner and were not contact-inhibited (Fig. 2A). Table 1 also shows the results when transfected cells were simultaneously selected for both geneticin-resistance and anchorage independence. Again, doubly selected colonies were obtained only after transfection with DNA containing the T24 gene and at least one enhancer, at about the same frequency as the morphologically distinct geneticin-resistant colonies obtained in liquid medium. This suggests that the morphological changes are characteristic of the second or completing transformation step, and it was confirmed by showing that cells from morphologically altered colonies plated with a high efficiency in semi-solid medium.

These results were not confined to hamster cells. Table 1 also shows the transformation of early passage rat cells from rat embryo and muscle and skin from 2-week-old Wistar rats. The results obtained were very similar to those observed with CHL(F3) cells. Geneticin-resistant colonies were obtained with all recombinant DNAs and all types of recipient cells and again the presence of transcriptional enhancers increased the frequency of geneticin-resistant colonies. However, the frequency was at least 10 times lower than that obtained with CHL(F3) cells (the results shown for rat cells in Table 1 were obtained with 1 μ g of recombinant DNA), confirmed by dose response tests (data not shown). The frequencies obtained following transfection of low passage lung and kidney cells from 2-week-old rats were even lower. Most importantly, morphologically altered cells and anchorage-independent colonies were obtained only after transfection with DNA containing the T24 gene and enhancers. Typical morphological appearances of normal cells and their transformed counterparts are shown in Fig. 2B. Transformation with pHO6N1 resulted in a significant number of geneticin-resistant colonies in liquid medium, but

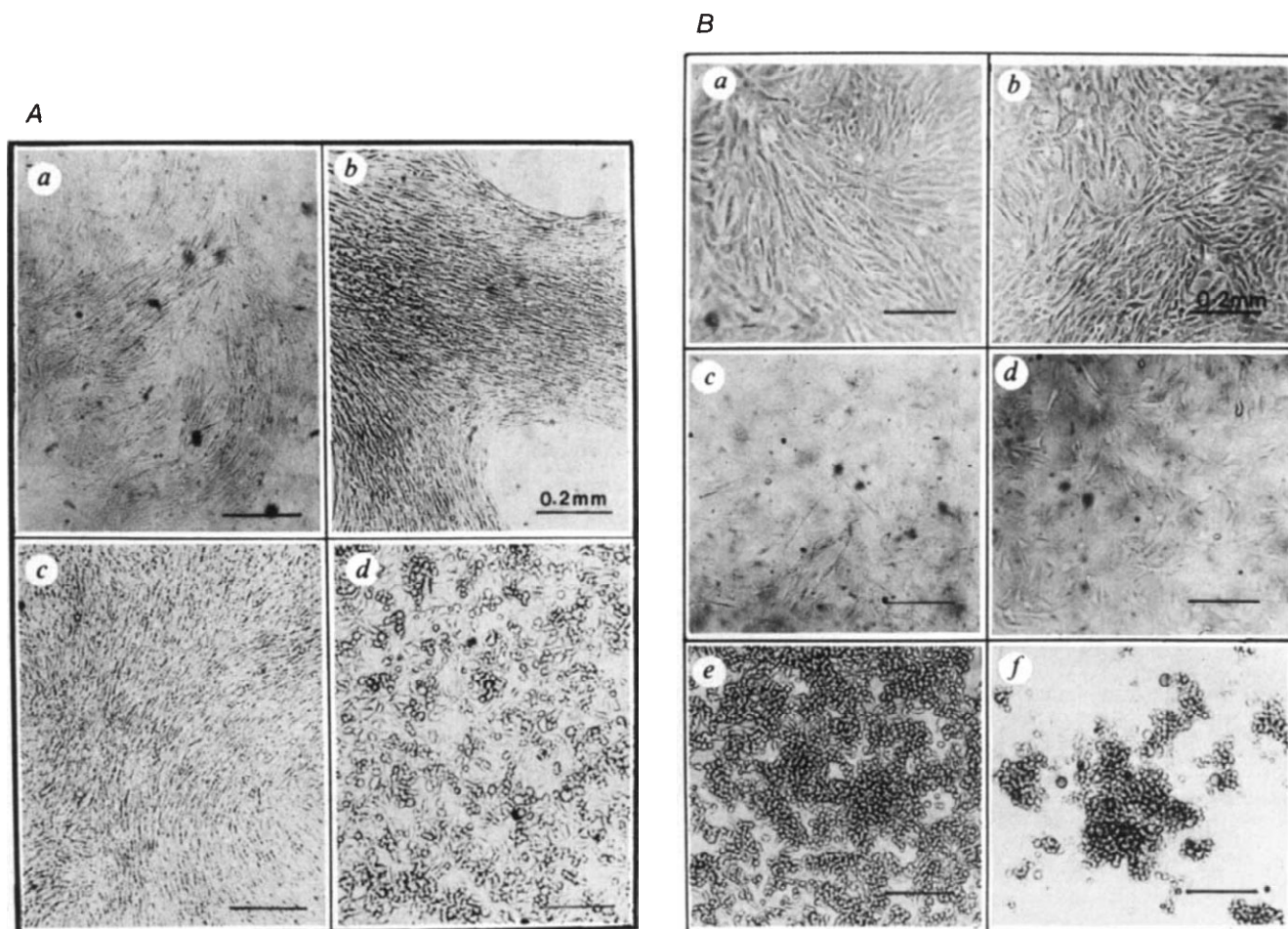


Fig. 2 A, Normal and transformed Chinese hamster lung cells: a, CHL(F3); b, FAGT-2; c, FHO6N1-1; d, FHO6T1-1 cells. B, Normal and transformed Wistar rat cells: a, second passage muscle cells; b, second passage skin cells; c, muscle cells transformed with pHO6N1; d, skin cells transformed with pHO6N1; e, muscle cells transformed with pHO6T1; f, skin cells transformed with pHO6T1.

these were morphologically normal. Simultaneous selection for geneticin resistance and growth in Methocel failed to yield any anchorage-independent colonies. Transfection with pAGT1 resulted in a very low number of geneticin-resistant colonies and no anchorage-independent cells. When cultures were transfected with pAGT1 or pHO6T1 and plated in Methocel in the absence of geneticin, numerous small anchorage-independent colonies appeared which rapidly senesced (200 colonies per 10^6 cells for pAGT1 and ≥ 500 per 10^6 cells plated for pHO6T1). Transfection of 2×10^5 rat cells in liquid culture with 20 μ g of plasmid pT24 (ref. 22), which contains the T24 oncogene without a biochemically selectable marker, followed by selection in low serum, resulted in the transient appearance of morphologically altered cells which subsequently senesced.

In summary, in both the hamster and rat cells the presence of the mutant T24 *ras* gene and transcriptional enhancers correlates with our ability to isolate directly morphologically altered, anchorage-independent cells with almost the same frequency as geneticin-resistant cells.

Phenotypic properties of cloned cells

Individual geneticin-resistant colonies of Chinese hamster and rat cells were picked and propagated *in vitro*. The phenotypes of individual colonies were analysed by determining which cells were rescued from senescence, which showed striking morphological changes and grew in semi-solid medium, and which induced tumours in nude mice (Table 2).

All of the geneticin-resistant colonies derived from vector plasmids pAG60 or Homer 6 (four out of four individual colonies tested in each case) senesced and were unable to form cell lines (Table 2). To test for tumorigenicity, 10–20 geneticin-resistant colonies from pAG60 or Homer 6 transfections were pooled to obtain sufficient cells, and inoculated into nude mice. No tumours were obtained. However, 7 out of 10 colonies tested from transfections on hamster cells with pAGT1, which contains the T24 gene but no known transcriptional enhancers, were rescued from senescence. These cells showed no striking morphological changes, were not anchorage independent and did not produce tumours in nude mice. In contrast, all of the tested geneticin-resistant colonies derived from pHO5T1, pATGM1, pHO6T1 and pHO6T2, in which the T24 gene is adjacent to either one or two enhancers, were morphologically altered, anchorage independent and produced tumours in nude mice. The tumours grew rapidly (the animals became moribund within 10 days) and on necropsy, metastatic infiltrations of other organs were observed in some cases. These results were repeated with Wistar rat cells transformed with pHO6T1 and pHO6T2 and strikingly, the anchorage-independent cells induced rapidly growing progressive tumours in nude mice (Table 2) and 3-week-old Wistar rats (data not shown). Very few geneticin-resistant colonies were obtained with pAGT1 and rat cells (see Table 1). Two such colonies were picked but failed to grow.

None of the geneticin-resistant colonies obtained from hamster or rat cells after transfection with recombinants containing the normal Ha-*ras*-1 gene were anchorage independent or able

Table 2 Phenotypes of geneticin-resistant hamster and rat cells

Cells	No. of clones	Transfected with	Rescued from senescence	Morphologically transformed	Anchorage independent	Tumorigenicity
Chinese hamster						
CHL(F3)	5	—	0/5	0/5	0/5	0/1*
FAG	4	pAG60	0/4	0/4	0/4	0/1*
FHO6	4	Homer 6	0/4	0/4	0/4	0/1*
FAGN1	2	pAGN1	0/2	0/2	0/2	0/1*
FAGT1	10	pAGT1	7/10	0/10	0/10	0/2
FHO6N1	10	pHO6N1	9/10	0/10	0/10	0/2
FHO6T1	4	pHO6T1	4/4	4/4	4/4	2/2
FHO6T2	4	pHO6T2	4/4	4/4	4/4	2/2
FHO5T1	4	pHO5T1	4/4	4/4	4/4	2/2
FATGM1	4	pATGM1	4/4	4/4	4/4	2/2
FATGM2	4	pATGM2	4/4	4/4	4/4	2/2
Rat						
Muscle	4	—	0/4	0/4	0/4	0/1*
Skin	4	—	0/4	0/4	0/4	0/1*
RMHO6	4	Homer 6	0/4	0/4	0/4	0/1*
RSKHO6	4	Homer 6	0/4	0/4	0/4	0/1*
RMHO6N1	4	pHO6N1	4/4	0/4	0/4	0/2
RSKHO6N1	4	pHO6N1	3/4	0/4	0/4	0/2
RMHO6T1	4	pHO5T1	4/4	4/4	4/4	2/2
RSKHO6T1	4	pHO6T1	4/4	4/4	4/4	2/2
RMHO6T2	4	pHO6T2	4/4	4/4	4/4	1/1
RSKHO6T2	4	pHO6T2	4/4	4/4	4/4	1/1

Individual colonies from the experiments shown in Table 1 were picked as described in Table 1 legend and propagated in SF12 liquid medium containing 15% Hyclone serum and 200 $\mu\text{g ml}^{-1}$ geneticin. Cells which grew for more than 100 cell doublings were classified as rescued from senescence. The classification of morphologically altered cells is described in Table 1 and Fig. 2 legends and anchorage independence was classified by a plating efficiency of >20% in the semi-solid medium described in Table 1. Tumorigenicity was tested by inoculating 1×10^6 cells subcutaneously into four or five 1-month-old nude mice. With tumorigenic cells lines, experimentally induced tumours appeared within 1 week in all inoculated animals, whereas no tumours were detected up to 2 months after inoculation of non-tumorigenic cells.

* In these cases insufficient numbers of cells from individual colonies were obtained for the tumorigenicity tests because the cells senesced. To obtain sufficient numbers the cells from 10–20 colonies were pooled and propagated for a limited number of passages before testing.

to produce tumours in experimental animals. However, a high proportion of the hamster colonies produced by pHO6N1 (9 out of 10) and pANGM1 (4 out of 4), where the normal gene is flanked by enhancers, were rescued from senescence and grew into established cell lines. The same was true for rat muscle or skin cells transformed with pHO6N1. Four out of four muscle and three out of four skin cell foci grew into established lines. In no case did we observe any obvious 'crisis' during the immortalization process. Geneticin-resistant colonies derived from transfections with pAGN1 or pAGN2, which contain no known enhancers, were very difficult to propagate. Only two colonies grew *in vitro* and both senesced after about 20 doublings.

Malignant conversion

FAGT1-2 and FHO6N1-1 are immortalized Chinese hamster cell lines obtained by transfection of CHL(F3) cells with recombinants pAGT1 and pHO6N1, respectively. These cells have a near normal morphology (see Fig. 2A), are contact-inhibited, anchorage dependent and are not tumorigenic (see Table 2). However, they have a $\sim 10^{-7}$ frequency of spontaneous transformation to anchorage independence, detected by plating in semi-solid media, and these anchorage-independent cells are tumorigenic (data not shown).

Table 3 shows the ability of various recombinants to convert these two lines to an anchorage-independent phenotype. As expected from the previous results, pHO6T1 can readily transform FAGT1-2 and FHO6N1-1 cells to anchorage independence. However, the frequencies obtained (7.6×10^{-3} to 1.7×10^{-2}) are much higher than that obtained with primary cells ($\sim 8 \times 10^{-5}$, Table 1). Transfection with R15²³, a clone which contains the adenovirus-2 Ela gene, also produces anchorage-independent colonies with both cell lines, although at a lower frequency. The R15 clone had no detectable effect on the anchorage independence of CHL(F3) cells (data not shown).

Plasmid pHO6N1, containing the normal gene in the presence of enhancers, also transformed both cell lines, as did pAGT1, containing the T24 gene with no enhancers. Thus, immortalized cells could be transformed to anchorage independence using the same donor plasmid as in the original immortalization step. Because no anchorage-independent cells could be obtained by transfection of 2×10^5 CHL(F3) cells with up to 20 μg of these donor DNAs, we suggest that this represents a qualitative change in immortalized cells. That is, the immortalization process results in qualitative changes to the cells which renders them more sensitive to further transformation even with the same gene construction. Presumably, conversion to anchorage independence with the same gene construction reflects a quantitative increase in the expression of that gene in the already immortalized cells. No further transformation of FAGT1-2 cells was obtained using pAGN1, which contained the normal gene with no enhancers, suggesting that conversion to anchorage independence requires either the mutation of the T24 gene or enhancer expression of the normal Ha-ras-1 gene. As Homer 6 produced no measurable increase in the frequency of anchorage-independent colonies, we assume that the enhancers in pHO6N1 act in a *cis* rather than a *trans* manner, probably at the level of transcription.

Combining the above results, we suggest that the transforming potential of the Ha-ras-1 gene is modulated at the level of transcription as well as mutation. In the absence of enhancers the mutant T24 gene can rescue cells from senescence, but cannot transform them to a tumorigenic phenotype. In the presence of enhancers acting in *cis*, the gene directly triggers events which result in a tumorigenic phenotype. A similar effect is observed with the normal Ha-ras-1. In the absence of enhancers, no rescue from senescence or complete transformation is obtained. In the presence of enhancers the normal gene is capable of rescuing cells from senescence. Apparently, both transcriptional activation and mutation are required to trigger tumorigenic conversion.

Expression of human Ha-ras-1

Selection of geneticin-resistant colonies after transfection with recombinant DNA ensures that all selected cells contain donor DNA. This was confirmed by Southern blot analysis (data not shown) which showed the presence of one to five copies of donor DNA, in some cases as full-length copies, in other cases individually integrated into host or carrier DNA.

The level of Ha-ras-1-specific transcripts in transformed cells was analysed by Northern blot and dot-blot analyses, using the 6.6-kb *Bam*HI fragment of the cloned T24 gene as a hybridization probe. The expected 1.2-kb Ha-ras-1-specific RNA was detected in most transformed cells. Much higher levels of the 1.2-kb RNA were detected in cells transformed with pHO6T1, containing two enhancers, than in two cell lines obtained with pAGT1, which contains no known enhancers. Moreover, when the pHO6T1 and pHO6T2 transformed cells were passaged in nude mice, the resultant tumour cells also contained high levels of the *ras* transcript (data not shown).

The results were confirmed by dot-hybridization tests (Fig. 3). Cells transformed with recombinants containing the T24 gene and no known enhancers (FAGT1-1 and FAGT1-2) contained relatively low amounts of Ha-ras-1-specific RNA (even lower than the human T24 bladder carcinoma cell line). In contrast, much higher levels of Ha-ras-1-specific RNA were found in cells transformed with recombinants which contained either the normal or mutant T24 genes plus known enhancers (FHO6N1-1, FHO6N2-1, FHO5T1-1, FHO5T1-2, FHO6T1-1 and FHO6T2-1). From Fig. 3 it can be estimated that these cells contained 20–60 times as much RNA as FAGT1-1 or FAGT1-2. Again, the high transcription level was maintained in tumour cells produced by passage in nude mice (FHO6T1-1T1 and FHO6T2-2T1). Similar results were obtained with transformed rat cells (data not shown).

Karyotypic analysis

Morphologically altered geneticin-resistant hamster and rat colonies were picked and the karyotypes analysed after 15–20 cell doublings. All of the transformed lines were subsequently shown to have a tumorigenic phenotype. As expected, the hamster CHL(F3) cells and the normal Wistar rat skin and muscle cells had a normal modal chromosome number (22 and 42, respectively). Three out of four tumorigenic hamster cells, isolated after transfection with pHO6T1 or pHO6T2, contained a near normal modal distribution, while the other was almost tetraploid. In contrast the transformed rat skin and muscle cells exhibited marked aneuploidy. On further passage the proportion of cells with a near normal number of chromosomes decreased and eventually most cells became heteroploid. These results suggest that the transformed cells were chromosomally abnormal. When, to investigate this in more detail, the banding patterns of the transformed hamster cells were determined, in each case a distinctive karyotype different from CHL(F3) cells was obtained and the presence of marker chromosomes noted (D.A.S., M. Freshney, R. Balfour and N.M.W., unpublished results).

Discussion

We have demonstrated here the potential for a single mutated human oncogene, the Ha-ras-1 from the T24 bladder carcinoma line, to trigger both step 1 (rescue from senescence) and step 2 (tumorigenic conversion) transformation of early passage rodent cells. Furthermore, we show that the 'normal' Ha-ras-1 proto-oncogene can rescue early passage cells from senescence. This transforming ability of the proto-oncogene, and the ability of the T24 gene to cause tumorigenic conversion, depends on the presence in the vector DNA of transcription enhancers. We suggest that the increased transforming potential of the two genes results from increased expression of the p21 proteins. Strong support for this suggestion comes from the experiments of Table 3, which show that the enhancer effect is in *cis* and

Table 3 Transformation of immortalized Chinese hamster lung cells to anchorage independence

Recipient cells	Immortalized with	Donor DNA	Colonies per plate per μ g DNA per 10^4 cells* (av. $\pm$ s.d.)
FAGT1-2	pAGT1	pHO6T1	139 $\pm$ 30
FAGT1-2	pAGT1	R15	22 $\pm$ 11
FAGT1-2	pAGT1	pHO6N1	80 $\pm$ 19
FAGT1-2	pAGT1	pAGT1	9 $\pm$ 6.2
FAGT1-2	pAGT1	pAGN1	0
FAGT1-2	pAGT1	Homer 6	0
FAGT1-2	pAGT1	Salmon	0
FHO6N1-1	pHO6N1	pHO6T1	92 $\pm$ 16
FHO6N1-1	pHO6N1	R15	13 $\pm$ 8.5
FHO6N1-1	pHO6N1	pHO6N1	50 $\pm$ 16
FHO6N1-1	pHO6N1	pAGT1	16 $\pm$ 6.5
FRO6N1-1	pHO6N1	Homer 6	0
FRO6N1-1	pHO6N1	Salmon	0

FAGT1-2 and FHO6N1-1 are cells rescued from senescence after transfection of CHL(F3) with plasmids pAGT1 and pHO6N1, respectively (see also Table 2).

* 1 μ g of each superhelical plasmid DNA mixed with 10 μ g salmon sperm DNA as carrier in 0.5 ml calcium phosphate DNA co-precipitate were added per 1×10^5 recipient cells in 5 ml medium per 25-cm² flask. 24 h later the medium was changed with 5 ml non-selective medium and incubation continued at 37 °C for 24 h. Cells were then trypsinized, counted and plated at 10-fold dilutions in 20 ml Methocel-containing medium per 9-cm plate in the presence of 200 μ g ml⁻¹ geneticin. Colonies were counted 8 days post-plating. The data are derived from the results of six plates per donor DNA from two experiments.

not in *trans*, and from those of Fig. 3, which show increased levels of Ha-ras-1 transcripts in cells transformed with enhancer-containing DNA.

These results are consistent with recent reports which show that the T24 gene readily transforms already immortalized Chinese hamster cells²⁴ and rat cells¹⁸, as well as the standard NIH 3T3 cell line^{7,8}. However, our data depart from recent claims that the T24 gene will only cause tumorigenic conversion of primary cells in combination with either the adenovirus *Ela* gene or the avian *v-myc* gene^{18,19}. While we agree that in the absence of enhancers the T24 gene may require transformation with other oncogenes to induce focus formation and tumorigenicity, our results clearly show that the T24 gene when linked to enhancers is able to trigger tumorigenic conversion on its own. We suggest that these differences reflect the levels of expression of the mutant T24 gene: at a low level of expression the presence of an additional gene is required; at high levels of expression the T24 gene alone suffices. Our recent data suggest that similar results can be obtained for the mutant N-ras gene²⁵ cloned from the HT1080 human fibrosarcoma cell line (unpublished results). It is therefore important to distinguish between quantitative and qualitative effects in attempting to define the effect of oncogenes in *in vitro* transformation systems.

Our inability to induce morphologically altered foci in liquid culture by transfection of early passage cells with the T24 gene in the absence of enhancers is consistent with the data of Ruley¹⁹ and Newbold and Overall²⁰ with early passage rat and Syrian hamster cells. However, like Newbold and Overall²⁰ and Land *et al.*¹⁸, we did observe anchorage-independent colonies which senesced, in the absence of geneticin selection. We believe that these apparently discrepant results can be explained by the transient expression and release of transforming growth factors (TGFs) in cultures transfected with *ras* genes (I. B. Pragnell, N.M.W. and D.A.S., unpublished results). These TGFs efficiently induce anchorage-independent phenotypes in already immortalized cells and we see no reason that they should not do the same in early passage cells, albeit at a lower frequency. In the presence of geneticin, these indirectly stimulated cells

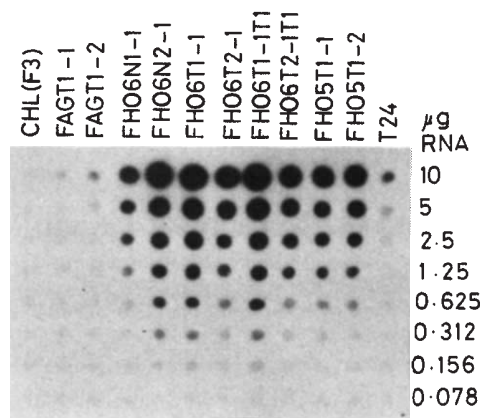


Fig. 3 An autoradiograph showing RNA spot-hybridization analysis of Ha-ras-1 transcripts present in recipient CHL(F3) and transformed with pAGT1 (FAGT1-1, FAGT1-2), pHO6N1 (FHO6N1-1), pHO6N2 (FHO6N2-1), pHO6T1 (FHO6T1-1), pHO6T2 (FHO6T2-1), pHO5T1 (FHO5T1-1, FHO5T1-2), tumours induced in nude mice by FHO6T1-1 (FHO6T1-1T1) or FHO6T2-1 (FHO6T2-1T1) and the T24 human bladder carcinoma cell lines. Total cell RNA extracted from cells as previously described²⁴ and twofold serial dilutions of 10 µg down to 0.078 µg were spotted onto nitrocellulose³⁵. The ³²P-labelled 6.6-kb *Bam*HI fragment carrying the T24 oncogene was used as a probe.

would not grow, and only cells containing activated *ras* genes stably expressing at high levels would form colonies.

While it is clear that the T24 gene in the absence of enhancers can immortalize CHL(F3) cells, the only two rat colonies we tested after transfection with this gene, rapidly senesced. Further experiments on a larger scale are required to determine if there is a qualitative difference between CHL(F3) cells and rat cells in this respect, apart from the quantitative differences in transformation frequencies observed between the two systems. Nonetheless, transfer of the normal Ha-ras-1 proto-oncogene in the presence of enhancers leads to immortalization of both CHL(F3) cells and Wistar rat cells. All eight rat cell geneticin-resistant colonies obtained with vectors pAG60 or Homer 6 and tested independently, senesced and failed to form established cell lines. After transfer of pHO6N1, 9 out of 10 CHL(F3) colonies and 7 out of 8 rat cell colonies grew into established cell lines.

It is of considerable interest that CHL(F3) cells immortalized with either the T24 gene without enhancers or the normal Ha-ras-1 gene in the presence of enhancers, can be converted to anchorage-independent cells with the same gene construction as used in the immortalization step (see Table 3). We interpret this to mean that unknown qualitative changes accumulate in

immortalized cells which render them very sensitive to further tumorigenic conversion, as anchorage-independent conversion of primary cells cannot be achieved even with large amounts of the same donor DNA. Interestingly, the adenovirus Ela gene behaves as a second-step conversion gene in immortalized Chinese hamster cells (Table 3), although it is usually considered to be an 'immortalization' gene¹⁸. Similarly, in our hands Ha-ras-1 can behave as an immortalization gene, although it is usually considered to be a late step or conversion gene¹⁸⁻²⁰. These results are consistent with those of Blair *et al.*²⁶ and Chang *et al.*²⁷, who showed the proto-oncogenes *c-mos* and Ha-ras-1 to have second-step transformation activity in NIH 3T3 fibroblasts when activated by transcriptional enhancers. Thus, a proto-oncogene and the equivalent activated oncogene can have both step 1 and step 2 transforming potential, depending on the assay system chosen.

It is of interest to attempt to relate our findings to *in vivo* carcinogenesis. Although there are few detailed reports on the expression of *ras* genes in pre-malignant lesions *in vivo*, we note that chemically induced benign papillomas of mouse skin contain a cellular Ha-ras gene which is active in transfection assays and gives rise to elevated transcript levels²⁸. Moreover, pre-malignant polyps of the human colon as well as adenocarcinomas have greatly elevated levels of *ras*-related transcripts compared with normal colonic mucosa²⁹. In the latter case it is not known whether transcription is from a mutant, activated gene, but the experiments provide support for the notion that *ras* genes may participate in early stages of the *in vivo* carcinogenic process, perhaps even in the establishment phase.

The mechanisms by which *ras* gene products effect transformation *in vitro* and *in vivo* are not understood. We emphasize that although our results suggest a quantitative aspect to *in vitro* transformation by the *ras* oncogene, they are completely consistent with multi-step models of carcinogenesis^{5,6}. Several events are required to reveal the most active transforming potential of Ha-ras-1 oncogenes. Although activated Ha-ras-1 can trigger the complete tumorigenic conversion of very early passage rodent cells *in vitro*, it seems likely that if similar mutational and transcriptional activation occurs *in vivo*, as yet unknown changes in other cellular genes may be necessary before the fully malignant cell emerges.

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Note added in proof: The introduction of plasmid pHO6T1 into primary cultures of adult Syrian hamster muscle cells resulted in the appearance of geneticin-resistant, anchorage-independent cells, which induced progressively growing tumours in nude mice.

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- Armitage, P. & Doll, R. *Br. J. Cancer* **11**, 161-169 (1957).
- Berenblum, I. *Cancer Res.* **1**, 807-814 (1941).
- Hayward, W. A., Neel, B. G. & Astrin, S. M. *Nature* **290**, 475-480 (1980).
- Cooper, G. M. & Neiman, P. E. *Nature* **292**, 857-858 (1981).
- Knudson, A. G. *Cancer Invest.* **1**, 187-193 (1983).
- Spandidos, D. A. *Anticancer Res.* **3**, 121-125 (1983).
- Cooper, G. M. *Science* **217**, 801-806 (1982).
- Weinberg, R. A. *Adv. Cancer Res.* **36**, 149-156 (1982).
- Rassoulzadegan, M. *et al. Nature* **300**, 713-715 (1982).
- Houweling, A., Van der Elsen, P. & van der Eb, A. J. *Virology* **105**, 537-550 (1980).
- Van der Eb, A. J. *et al. Cold Spring Harb. Symp. quant. Biol.* **44**, 383-399 (1980).
- Triesman, R., Novak, V., Favoloro, J. & Kamen, R. *Nature* **292**, 595-600 (1981).
- Weiss, R. A., Teich, N. M., Varmus, H. & Coffin, J. M. *The Molecular Biology of Tumor Viruses—RNA Tumor Viruses* (Cold Spring Harbor Laboratory, New York, 1982).
- Duesberg, P. H. *Nature* **304**, 219-226 (1983).
- Vande Woude, G. F., Levine, A. J., Topp, W. C. & Watson, J. D. *Cancer Cells*, Vol. 2 (Cold Spring Harbor Laboratory, New York, in the press).
- Littlefield, J. W. *Science* **218**, 214-215 (1982).
- Pollack, R. E. *Science* **218**, 1069-1070 (1982).
- Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304**, 596-602 (1983).

- Ruley, H. E. *Nature* **304**, 602-606 (1983).
- Newbold, R. F. & Overell, R. W. *Nature* **304**, 648-651 (1983).
- Graham, F. L. & Van der Eb, A. J. *Virology* **52**, 456-461 (1973).
- Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. *Nature* **298**, 343-347 (1982).
- Stow, N. D. *Nucleic Acids Res.* **10**, 5105-5119 (1982).
- Sager, R., Tanaka, K., Lau, C. C., Ebend, Y. & Anisowicz, A. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7601-7605 (1983).
- Hall, A., Marshall, C. J., Spurr, N. K. & Weiss, R. A. *Nature* **303**, 396-400 (1983).
- Blair, D. G. *et al. Science* **212**, 941-943 (1981).
- Chang, E. H., Furth, M. E., Scolnick, E. M. & Lowy, D. R. *Nature* **297**, 479-483 (1982).
- Balmain, A., Ramsden, D. M., Bowden, G. T. & Smith, J. *Nature* **307**, 658-660 (1984).
- Spandidos, D. A. & Kerr, I. B. *Br. J. Cancer* **49**, 681-688.
- Pulciani, S., Santos, E., Louver, A. V., Long, L. K. & Barbacid, M. *J. Cell Biochem.* **20**, 51-61 (1982).
- Colbere-Garapin, F., Horodniceanu, F., Kourilsky, P. & Garapin, A. C. *J. molec. Biol.* **150**, 1-14 (1981).
- Lang, J. C., Wilkie, N. M. & Spandidos, D. A. *J. gen. Virol.* **64**, 2679-2696 (1983).
- Dhar, R., McClements, W. L., Enquist, L. W. & Vande Woude, G. F. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3937-3941 (1980).
- Spandidos, D. A. & Paul, J. *EMBO J.* **1**, 15-20 (1982).
- Spandidos, D. A., Harrison, P. R. & Paul, J. *Biosci. Rep.* **1**, 911-920 (1981).

Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse

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An infectious retrovirus vector has been used to transfer a bacterial gene encoding resistance to the neomycin analogue G418 into pluripotent haematopoietic stem cells present in explanted murine bone marrow tissue. Subsequent transplantation of the cells into lethally irradiated mice results in engraftment of the animals with donor haematopoietic tissue containing the bacterial gene. This approach affords an efficient and rapid means of re-introducing genetically modified tissue into intact organisms and provides a system whereby the expression and regulation of cloned genes can be followed within the context of a well characterized developmental programme.

THE introduction of genes into murine haematopoietic tissue offers many of the advantages of both the *in vitro*¹⁻⁴ and *in vivo*⁵ gene transfer systems currently available. Haematopoiesis in the mouse is a unique developmental programme involving well characterized cell lineages comprising pluripotent stem cells, progenitor cells and mature end cells^{6,7}. A variety of *in vitro* and *in vivo* assays exist for identifying members of the haematopoietic lineages⁸⁻¹⁰, and syngeneic bone marrow transplantation^{7,11} offers unique opportunities for re-introducing functional bone marrow tissue into mice after various *in vitro* manipulations.

It would be particularly useful to be able to introduce genes into the pluripotent haematopoietic stem cells (CFU-S)¹¹ which give rise to all blood tissues¹². However, because CFU-S is a very minor component of mouse bone marrow (0.01%)¹¹ that has not been defined morphologically, and because of the inefficiency of standard gene transfer methods, this has not been easy^{13,14}. Here we describe the successful use of retrovirus vectors^{15,16} to transfer new genes efficiently into haematopoietic stem cells, and the use of bone marrow transplantation to engraft mice with the genetically modified haematopoietic cells.

General strategy

It is theoretically possible with highly transmissible retrovirus vectors to introduce genes into 100% of recipient bone marrow cells. Accordingly, in one of our protocols, freshly explanted bone marrow tissue is simply infected with recombinant virus and subsequently used to transplant lethally irradiated recipients. Alternatively, bone marrow cells cultured in conditions in which active CFU-S cell division is known to occur^{17,18} are used as recipients for gene transfer. Successful gene transfer is monitored by Southern blot analysis of genomic DNA extracted from spleen, a major site of haematopoiesis in the transplanted animal (Fig. 1). Evidence for successful infection of CFU-S can be obtained from analysis of spleen DNA (because cells in the spleen are derived almost exclusively from donor CFU-S¹⁹) and from serial transplantation of infected single 12-14-day colonies derived from CFU-S into secondary recipients²⁰.

Infection and transplantation

Bone marrow cells were infected *in vitro* using the two methods described above. The source of virus for these experiments was a cell line termed ψ 2 MSV DHFR-NEO (C. Cepko and R.C.M., unpublished), chosen principally because it produces unusually high titres of virus ($>10^7$ colony-forming units (CFU) per ml) free of wild-type virus. Pure stocks of infectious recombinant

virus were derived from a specialized cell line, termed Psi-2 (ψ 2), capable of generating infectious recombinant virus yet deficient in the ability to produce any detectable wild-type virus²¹. Figure 2 shows the structure of the MSV *dhfr-neo* genome. Briefly, the genome is derived from a Moloney murine sarcoma virus genome²² and contains substitutions of viral sequences with both a mouse cDNA sequence encoding dihydrofolate reductase (DHFR)²³ and a DNA segment derived from the transposon Tn5 which encodes neomycin resistance in bacteria and G418 resistance in mammalian cells²⁴ (see Fig. 2 legend for details of structure).

The first method of infection involved 48 h co-cultivation of bone marrow cells from C3H/HeJ male mice with ψ 2 MSV DHFR-NEO, followed by intravenous (i.v.) injection of the non-adherent cells (50 CFU-S per mouse) into lethally irradiated syngeneic recipients. With the dose used to irradiate recipients, no endogenous spleen colonies were produced in recipients not given donor cells and all such animals died from bone marrow aplasia at 10-14 days post-irradiation. In the second method, bone marrow cells from CD-1 female mice were flushed into flasks containing a monolayer of ψ 2 MSV DHFR-NEO and cultured in conditions originally described by Dexter¹⁷ and modified by Mauch *et al.*¹⁸. After 6 days in culture, the non-adherent cells were collected and injected i.v. into lethally irradiated syngeneic recipients (200 CFU-S per mouse). The frequency of CFU-S was monitored after both infection methods and found to be 1-10 CFU-S per 10^5 bone marrow cells ($\sim 20\%$ of the frequency in freshly explanted marrow). Animals transplanted with appropriate numbers of cells (>50 CFU-S per mouse) have remained alive and healthy 4 months post-transplantation. Cells from regenerating spleens of transplanted animals were able to form 5-20 granulocyte-macrophage (CFU-GM) colonies *in vitro* per 10^5 cells; 1-2 burst-forming units-erythroid (BFU-E) colonies *in vitro* per 10^6 cells gave rise to 12-14-day spleen colonies in lethally irradiated secondary recipients (CFU-S) and differentiated into multiple haematopoietic lineages *in vivo* (see below).

Efficiency of transformation

As the spleen is a major site of haematopoiesis in the transplanted mouse, and is repopulated exclusively with donor CFU-S and more differentiated cell types derived from these transplanted CFU-S, we examined spleen DNA for the existence of recombinant viral genomes. Splenectomies were performed on mice 10-14 days after transplantation and the cells obtained were used to prepare high molecular weight DNA as described in Fig. 3 legend. The DNA was cleaved with *Sac*I, an enzyme that cuts once within the long terminal repeats (LTRs) of the

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recombinant genome, thus excising the integrated provirus (Fig. 2). Electrophoretically fractionated DNA was blotted and the nitrocellulose probed for neo-pBR hybridizing sequences by the method of Southern²⁵. Figure 3 shows that the expected 3.8-kilobase (kb) *neo* hybridizing fragment is present in all mice transplanted with bone marrow cells infected by either 48 h co-cultivation of 6 days' co-cultivation with the modified Dexter

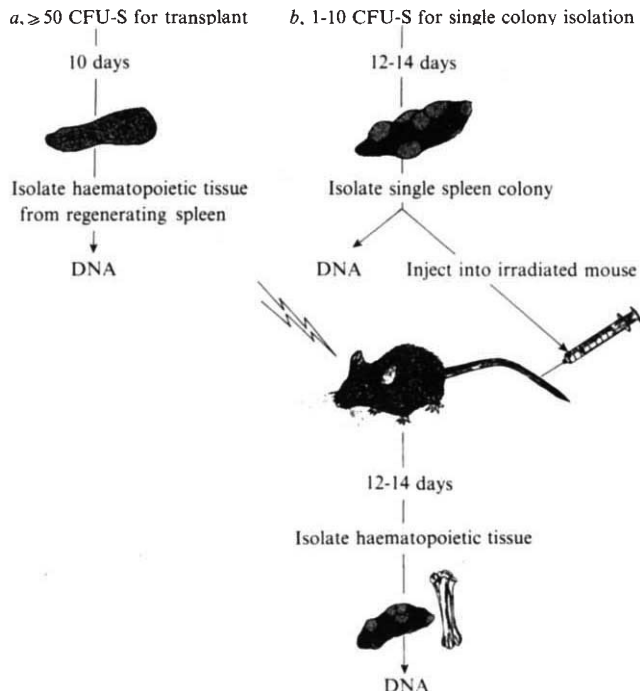


Fig. 1 General strategy for infection of bone marrow cells and transplantation into mice of ≥ 50 (a) and 1–10 (b) CFU-S for transplant and single-colony isolation, respectively.

Methods: The first method of infection involved the isolation of bone marrow cells from 8–12-week-old male C3H/HeJ (Jackson Laboratories) hind limbs. The tibiae and fibulae were flushed with 5 ml of α -media (Gibco) containing 10% calf serum (Gibco), penicillin (100 U ml⁻¹) and streptomycin (100 μ g ml⁻¹) and single-cell suspensions were made by triturating several times with a 5-ml pipette. 5×10^6 bone marrow cells were placed on a monolayer of ψ 2 MSV DHFR-NEO producer cells seeded at 1×10^6 cells per 100-cm² tissue culture dish, in α -media containing Polybrene (2 μ g ml⁻¹; Aldrich). These dishes were incubated for 48 h at 37 °C in a 5% CO₂ atmosphere. The non-adherent cells were then collected, counted and 1×10^6 – 5×10^6 cells containing 50 CFU-S were injected i.v. into lethally irradiated 8–12-week old syngeneic mice (1,100 rad, Cs source in two split doses at 107 rad min⁻¹). In the second method of infection, the contents of one 8–12-week-old female CD-1 (Charles River) hind limb were flushed into a flask (Falcon 25 cm² surface area) using Fischer's modified mouse leukaemic cell media (Gibco) containing 20% horse serum (Gibco), 10^{-5} M hydrocortisone (MSD), penicillin (100 U ml⁻¹), streptomycin (100 μ g ml⁻¹) and Polybrene (2 μ g ml⁻¹). A monolayer of ψ 2 MSV DHFR-NEO had been established (5×10^5 cells per flask) in the flask 24 h before charging with bone marrow. The mixture of bone marrow and virus-producing cells was incubated at 33 °C, 7% CO₂ for 6 days. The non-adherent cells were collected, counted and 3×10^6 – 1×10^7 cells containing 200 CFU-S were injected i.v. into lethally irradiated CD-1 recipients (1,050 rad, Cs source in two split doses at 107 rad min⁻¹). **a.** Beginning at 10 days post-injection, haematopoietic cells were collected from the spleens of recipient mice by stripping the capsule free of all colonies in minimal essential medium (α -media). Single-cell suspensions were made and the cells counted and used to prepare DNA. **b.** Single-colony transfers were accomplished by injection of low numbers of cells (1–10 CFU-S) into lethally irradiated recipients. This procedure produced discrete haematopoietic spleen colonies in the recipient mice. At 12–14 days post-transplantation, the colonies were dissected under a microscope, counted and one-half of the cells were injected into a second lethally irradiated syngeneic mouse (see Fig. 5 legend). The remaining cells from the colony were used to prepare high molecular weight DNA.

culture, and that 10–25% of the haematopoietic cells from regenerating spleen bear the neo-pBR hybridizing fragment, assuming a single integration site per infected cell. Thus, the recombinant genome is transferred intact to a significant fraction of donor haematopoietic cells.

To investigate the number of distinct sites of integration, which depends in part on the number of different stem cells infected, the same DNA samples were cleaved with *AhaIII*, an enzyme that does not cut the proviral genome, and probed with the labelled neo-pBR sequences. Each hybridizing band represents a unique integration site. Figure 4 indicates the presence of multiple sites of integration, although the number varies considerably. Of particular interest is the variation in intensity of different hybridizing bands, which may reflect either the inherent variability in CFU-S colony size or the derivation of multiple CFU-S colonies from an initially infected CFU-S with high self-renewal capacity (see below). The appearance of multiple spleen colonies derived from the same CFU-S has, in fact, been reported previously²⁶. Furthermore, the number of integration sites observed seems to vary qualitatively between experiments (Fig. 4, compare lanes 1–3 with 4–6), even though the same infection protocol was used each time.

To determine the efficiency of gene transfer into CFU-S more directly, we examined DNA extracted from isolated single CFU-S colonies for the presence of neo-pBR hybridizing sequences (Fig. 1b). Lethally irradiated recipients were injected with small numbers of infected bone marrow cells (~ 1 –10 CFU-S) to generate discrete macroscopically visible CFU-S colonies in the spleen 14 days post-transplantation. Twenty individual colonies from five mice were dissected under a microscope and the resulting cells resuspended and counted. High molecular weight DNA was prepared from half the cells and analysed for the presence of the recombinant genomes. Southern blot analysis indicated that at least four of the colonies examined were neo-pBR-positive (data not shown). Interestingly, this analysis also indicated that most of the neo-pBR-positive CFU-S colonies contained multiple sites of integration, suggesting that CFU-S cells can be efficiently infected.

Serial transplantation

To demonstrate self-renewal in the infected CFU-S, we performed serial transplantations of single CFU-S colonies obtained after a primary transplant. Transplantation of cells from a neo-pBR-positive CFU-S should result in haematopoiesis in a secondary recipient and the haematopoietic tissue should

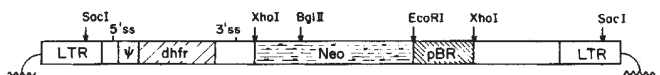


Fig. 2 Structure of the MSV DHFR-NEO genome. The derivation of the Moloney MSV vector backbone has been described in detail elsewhere¹⁵. Briefly, the sequences of the Moloney murine sarcoma virus genome that have been retained in the vector include the LTRs, sequences involved in the reverse transcription process and sequences necessary for the efficient encapsidation of the viral genome²¹ (designated ψ). The 5' splice site used in the generation of Moloney MLV *env* message is designated 5' ss. The retroviral *gag-pol* sequences have been replaced by a wild-type mouse cDNA encoding dihydrofolate reductase²³. Because the Moloney MSV genome lacks the 3' ss signals necessary for generation of spliced *env* transcripts, sequences encoding the splicing signal have been re-introduced into the vector as shown. Adjacent to a 1.4-kb DNA fragment encoding neomycin and G418 resistance in bacterial and mammalian cells, respectively²³ (labelled neo), there is a small *FnuDII* fragment of pBR322 encoding the plasmid origin of replication. The total size of the MSV DHFR NEO genome is 5.1 kb. Restriction endonuclease cleavage sites are as shown. *AhaIII* does not cleave the recombinant viral genome. The ψ 2 line harbouring the genome produces a titre of $>10^7$ G418-resistant colonies per ml of culture fluid on 3T3 cells. The recombinant genome that is transmitted contains a deletion of a majority of DHFR sequences (C. Cepko, unpublished).

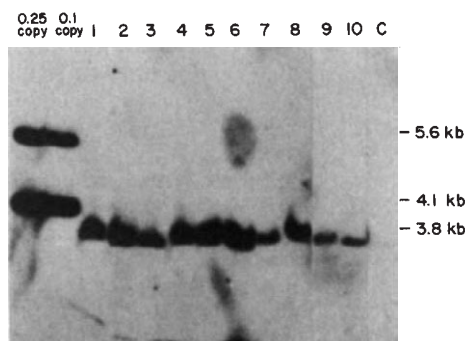


Fig. 3 Detection of recombinant proviral sequences in the DNA of transplanted haematopoietic cells. Spleen DNA was isolated from transplanted mice 10–14 days post-injection by detergent lysis, proteinase-K digestion and exhaustive extraction with phenol/chloroform. The resultant DNA was concentrated by ethanol precipitation and rehydrated in Tris-EDTA buffer or alternatively by extraction with butanol followed by dialysis into buffer. Restriction digests were performed using conditions specified by the manufacturer. Usually, 20 µg of cellular DNA were digested with a severalfold excess of endonuclease. Completion of each digestion was monitored by removing 10% of the reaction volume (after addition of enzyme) and adding it to 0.5–1.0 µg of bacteriophage λ DNA. Following parallel incubation, the reactions containing phage DNA were analysed electrophoretically for the spectrum of DNA fragments diagnostic of complete cleavage with a particular restriction enzyme. The large reactions, judged to be complete, were fractionated on agarose gels and transferred to nitrocellulose by the method of Southern²⁵. The transferred spleen DNA digests were probed for the presence of neo-pBR hybridizing sequences by hybridization with a ³²P-labelled DNA fragment as previously described³⁰. The probe encompassed sequences from the *Bgl*II site near the 5' end of the *neo* gene to the *Xho*I site flanking the 3' border of the pBR322 replication origin (see Fig. 2). Efficiency of marrow infection with the recombinant virus was estimated by inclusion of quantities of the DHFR-NEO plasmid DNA calculated to represent approximately 0.1 and 0.25 copies of *neo* sequence per genome within each blot. Spleen DNA samples were digested with *Sac*I. This enzyme cleaves once in the LTRs flanking the inserted proviral sequence, thus excising a 3.8-kb proviral DNA fragment (see Fig. 2). Lane C represents a negative control spleen DNA obtained from transplantation of marrow co-cultivated with ψ2 cells harbouring no recombinant genome. Spleen DNAs derived from two separate 48-h co-cultivations of fresh bone marrow with the ψ2 DHFR-NEO producer cell line are shown in lanes 1–6. Lanes 7–10 are spleen DNAs derived from marrow co-cultivated for 6 days in Dexter-like conditions. Two separate experiments (lanes 7, 8 and 9, 10) are shown. The larger 5.6-kb hybridizing fragment in the reconstruction lanes is due to the presence of the pBR322 replication origin in the labelled probe and the presence of a plasmid backbone in the DNA used for the copy number standards.

harbour the proviral sequences. X-ray irradiation-induced cytological markers have previously shown the clonality of individual CFU-S colonies²⁷. In the present studies, the generation of distinct proviral integration sites resulting from the infection of CFU-S provides a means of directly tracking the fate of the stem cells by Southern blot analysis.

To examine the self-renewal capacity of the four colonies found to harbour the recombinant genome, cells from three of the colonies were injected into secondary lethally irradiated recipients as described above. Inspection of the recipient spleens 14 days later showed that two of the three recipients had been engrafted with 8–10 CFU-S; the remaining recipient spleen contained no colonies. DNA was prepared from the spleen and bone marrow of all three mice and, along with DNA from the original colonies used for the transplantation, was cleaved with *Eco*RI and blotted to nitrocellulose. The blot was probed with neo-pBR sequences. *Eco*RI was used rather than *Aha*III so as to generate a band pattern more diagnostic of a particular

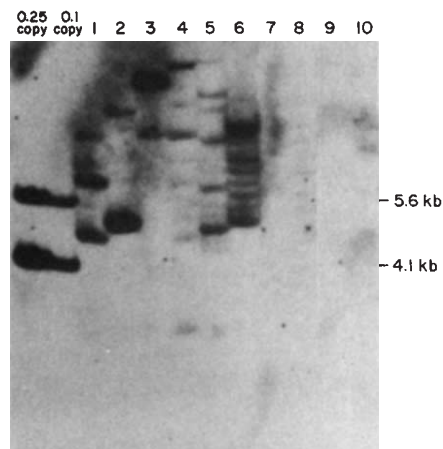


Fig. 4 Integration patterns of transplanted haematopoietic tissue. Lanes 1–6, digests of spleen DNA from the 48-h co-cultivation experiments; lanes 7–10, the proviral integration patterns resulting from 6-day co-cultivation experiments. Because of the low level of gene transfer in these latter experiments, prominent patterns are only observed within lanes 7 and 8. Other experiments reveal similar integrated proviral genomes in the DNAs represented in lanes 9 and 10 (data not shown). The DNA samples shown in Fig. 3 were digested with *Aha*III, which does not cleave within the proviral sequences, and probed with the same fragment described in Fig. 3. Each *neo* hybridizing DNA fragment visible in an *Aha*III digest blot constitutes a separate proviral integrant. All DNA samples are in the same order in this blot as in Fig. 3.

integration site (see Fig. 2). Figure 5 shows that haematopoietic cells (from both bone marrow and spleen colonies) from the two secondary recipients showing evidence of engraftment contained the same pattern of proviral integration as was found in the original primary colony used for the secondary transplant. Interestingly, the pattern of integration in both recipients was also identical, indicating that both donor colonies were probably derived from the same initially infected CFU-S. The presence of three hybridizing bands in each spleen and bone marrow DNA was unexpected, as each unique site of proviral integration should yield two hybridizing bands. Because digestion of the samples was judged to be complete (Fig. 2 legend), we speculate that one of the flanking *Eco*RI sites was partially resistant to cleavage, through DNA methylation of the C nucleotide of the *Eco*RI recognition sequence. Other workers have observed resistance of *Eco*RI sites containing a methylated C nucleotide (R. White, personal communication).

Cleavage of the secondary spleen DNA with *Sac*I, *Aha*III, *Bst*XI, *Hpa*I, *Sca*I (the latter four enzymes do not cleave the proviral genome) (Fig. 5b), *Apa*I, *Hind*III, *Bam*HI and *Bgl*II (data not shown) indicates unequivocally a single site of integration. A rough comparison of band intensities to copy number standards reveals that, as expected, most of the DNA derived from spleen cells in secondary transplants harbours the proviral sequence. The observation that cytocentrifuge preparations of spleen cells from both of these secondary recipients show the presence of erythroid precursors as well as mature granulocytes (Fig. 6) indicates that the infected stem cells exhibit self-renewal and multipotentiality.

Future prospects

The present experiments demonstrate the feasibility of introducing exogenous DNA sequences into the entire spectrum of haematopoietic tissues present in the mouse. Perhaps the major conclusion from these studies is that it is possible to introduce efficiently exogenous DNA sequences into pluripotent haematopoietic stem cells. Evidence for the infection of this cell type was obtained in several ways. First, DNA isolated from the spleens of recipients engrafted with bone marrow cells infected *in vitro* with recombinant retrovirus stocks was shown

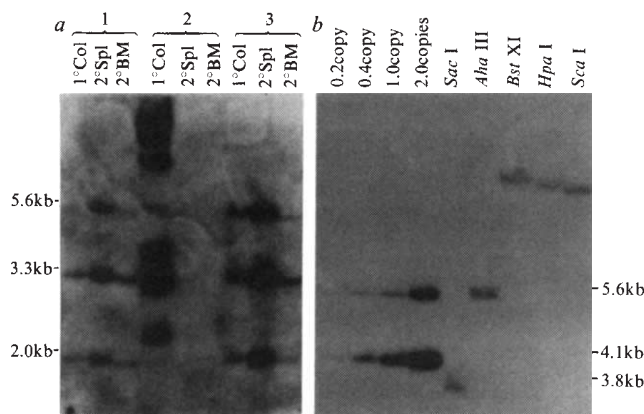


Fig. 5 Clonal transfer of infected haematopoietic stem cells to a second recipient mouse. *a*, DNA was prepared from spleen and bone marrow of each secondary recipient and, with the original colony DNA, was cleaved with *EcoRI*, which was chosen so as to produce a more complex band pattern diagnostic of a particular proviral site of integration (each site of integration should yield two hybridizing bands, see Fig. 2). The DNA was electrophoresed, transferred to nitrocellulose and the filter hybridized with ^{32}P -labelled neo-pBR sequences as described in Fig. 3 legend. The tracks are labelled according to the primary colony number (1, 2 or 3). 1° Col indicates the primary colony. 2° Spl samples are derived from the secondary spleens and 2° BM samples are derived from bone marrow of secondary recipients. *b*, Spleen DNA from secondary recipient 3 was further cleaved with *SacI*, *AhaIII*, *BstXI*, *HpaI* and *ScaI*, as shown, blotted and probed as above. Genome equivalents were generated by dilution of MSV DHFR-NEO DNA.

Methods: Freshly explanted bone marrow was co-cultivated for 48 h with the $\psi 2$ DHFR-NEO virus producer cell line as described in Fig. 1 legend. Injection of small numbers of the co-cultivated marrow cells resulted in 5–10 discrete spleen colonies 12–14 days after introduction into the irradiated host. Individual spleen colonies were isolated under a dissection microscope, taking care that each nodule was discrete and, by microscopic examination, not a conglomeration of several individual CFU-S colonies. In total, 20 such colonies were obtained from five transplanted animals and processed to yield a single cell suspension in α -medium by teasing with the aid of needles. Commonly, 10^6 – 10^7 cells were obtained per colony. The exclusive donor origin of these cells was inferred by the absence of any viable cells in a 12–14-day post-irradiation spleen obtained from a non-transplanted animal. The cell suspensions were divided into two aliquots. DNA was isolated from one aliquot, while the cells from the second aliquot were injected into a second irradiated mouse. Southern blot analysis of the single colony DNAs indicated that four of the colonies harboured recombinant proviral genomes (data not shown). Spleens from three secondary recipients transplanted with cells from three of the four *neo*-positive colonies were inspected for the presence of CFU-S at day 14 post-transplant. Two of the recipients contained 8–10 colonies; the third contained no colonies.

to contain proviral DNA sequences. This result alone is significant, as the spleen is a major site of haematopoiesis in transplant recipients and has been shown exclusively to harbour donor CFU-S and more differentiated members of the haematopoietic lineage derived from donor CFU-S²⁶. In addition, transplantation of cells derived from single CFU-S colonies harbouring the exogenous sequences in unique chromosomal locations led to the repopulation of secondary recipients with CFU-S containing the new sequences in those same chromosomal locations. Furthermore, morphological studies indicated that the transduced stem cells could differentiate in multiple lineages.

A key to the success of our experiments was the use of infectious retrovirus vectors to effect gene transfer to a substantial fraction of CFU-S in normal bone marrow cells, thus obviating the need for selective procedures to increase the proportion of transduced stem cells. Southern blot analyses of isolated single colonies suggest that the efficiency of gene transfer into

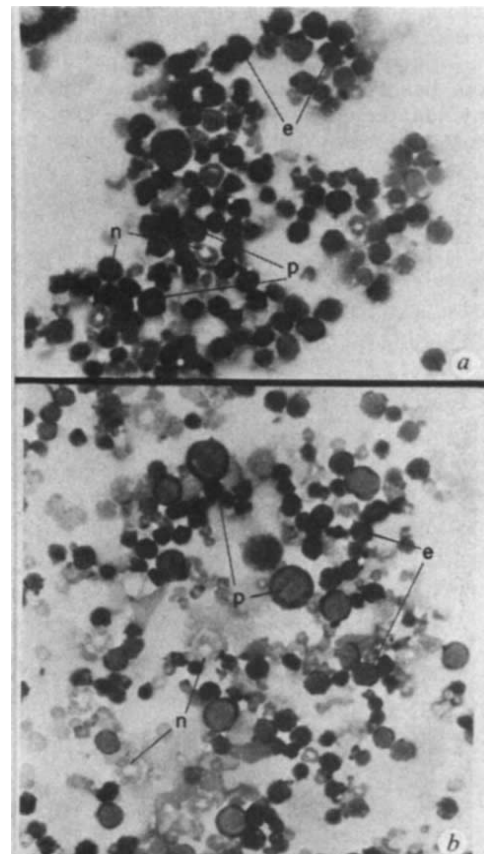


Fig. 6 Wright-Giemsa stain of haematopoietic tissue from day 14 secondary spleen colonies. An aliquot of cells obtained from the two secondary CFU-S colonies shown to harbour proviral sequence (Fig. 5) was centrifuged onto glass slides using a Shandon cytocentrifuge (1,250 r.p.m., 10 min) and subsequently stained with Wright-Giemsa using standard techniques. *a*, Secondary spleen colony cells derived from primary colony 1. *b*, Secondary spleen colony cells derived from primary colony 3. Specific haematopoietic cell types have been identified by morphology and labelled as follows: n, neutrophils; p, proerythroblast; e, erythroblasts.

CFU-S can approach 20%. However, what limits the efficiency of transduction is presently unclear. Viral titres are not likely to be limiting, as we have observed multiple integrations in most of the CFU-S-derived colonies examined. We suspect that the virus may be integrating only in a restricted subpopulation of CFU-S, since although recipients are routinely injected with more than 50 CFU-S, we find in some cases a relatively few distinct proviral integration sites. Assuming retroviruses integrate into chromosomal DNA in random locations²⁸, this finding might suggest that the population of CFU-S that can be infected is a restricted one, but necessarily one with high self-renewal capacity. Furthermore, since the number of integration sites varies even between experiments, we speculate that slight alterations in culture conditions or methods of infection may significantly alter stem cell cycle kinetics and therefore susceptible targets. Experiments are in progress to investigate this question.

Finally, we are aware of the inevitable parallels that will be drawn between the work described here and proposed scenarios for gene therapy in man²⁹. Ultimately, the specific parameters important for successful use of this technology to treat human diseases need to be examined. The efficiency of gene transfer into human haematopoietic stem cells, the regulation of genes introduced into haematopoietic tissue via retrovirus infection, and the safety and long-term stability of retroviral-mediated transformation are just a few of the factors needing extensive

study before a rational discussion of the feasibility of gene therapy in man. We hope we have helped to provide a suitable framework for addressing these issues.

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- Chao, M. V., Mellon, P., Charnay, P., Maniatis, T. & Axel, R. *Cell* **32**, 483–493 (1983).
- Zinn, K., Mellon, P., Ptashne, M. & Maniatis, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4897–4901 (1982).
- Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717–728 (1983).
- Huang, A. L., Ostrowski, M. C., Berard, D. & Hager, G. L. *Cell* **27**, 245–255 (1981).
- Palmiter, R. D., Norstedt, G., Gelfinas, R. E., Hammer, R. E. & Brinster, R. L. *Science* **222**, 809–814 (1983).
- Cline, M. J. & Golde, D. W. *Nature* **277**, 177–181 (1979).
- Nathan, D. G. & Oski, F. A. (eds) *Hematology of Infancy and Childhood* (Saunders, Philadelphia, 1981).
- Ferrero, D. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4114–4118 (1983).
- Johnson, G. R. *J. cell. Physiol.* **103**, 371–383 (1980).
- McLeod, D. L., Shreeve, M. M. & Axelrad, A. A. *Blood* **44**, 517–534 (1974).
- Till, J. E. & McCulloch, E. A. *Radiat. Res.* **14**, 213–222 (1961).
- Lewis, J. P. & Trobaugh, F. E. Jr *Nature* **204**, 589–590 (1964).
- Cline, M. J. et al. *Nature* **284**, 422–425 (1980).
- Carr, F., Medina, W. D., Dube, S. & Bertino, J. R. *Blood* **62**, 180–185 (1982).
- Mulligan, R. C. *Experimental Manipulation of Gene Expression* (ed. Inouye, M.) 155–173 (Academic, Orlando, Florida, 1983).

- Cepko, C. & Mulligan, R. C. *Cell* (in the press).
- Dexter, T. M., Allen, T. D. & Lajtha, L. G. *J. cell. Physiol.* **91**, 335–344 (1976).
- Mauch, P., Greenberger, J. S., Botnick, L., Hannon, E. & Hellman, S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2927–2930 (1980).
- Curry, J. L. & Trentin, J. J. *Dev. Biol.* **15**, 395–413 (1967).
- Magli, M. C., Iscove, N. N. & Odartchenko, N. *Nature* **245**, 527–529 (1982).
- Mann, R., Mulligan, R. C. & Baltimore, D. *Cell* **33**, 153–159 (1983).
- Van de Woude, G. F. et al. *Cold Spring Harb. Symp. quant. Biol.* **44**, 735–745 (1979).
- Nunberg, J. H., Kaufman, R. J., Chang, A. C. Y., Cohen, S. N. & Scrimgeour, R. J. *Cell* **19**, 355–364 (1980).
- Southern, P. J. & Berg, P. *J. molec. appl. Genet.* **1**, 327–341 (1982).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Barnes, D. W. H., Evans, E. P., Ford, C. E. & West, B. J. *Nature* **219**, 518–520 (1968).
- Wu, A. M., Till, J. E., Siminovich, L. & McCulloch, E. A. *J. cell. Physiol.* **69**, 177–184 (1967).
- Weiss, R., Teich, N., Varmus, H. & Coffin, J. (eds) *Molecular Biology of Tumor Virus—RNA Tumor Virus* (Cold Spring Harbor Laboratory, New York, 1982).
- Hearings before the Subcommittee on Investigations and Oversight of the Committee on Science and Technology U.S. House of Representatives, 97th Congress, 170 (US Government Printing Office, Washington, 1983).
- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).

LETTERS TO NATURE

A quantum mechanical explanation for Hund's multiplicity rule

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Hund's multiplicity rule, according to which a high spin state has a lower energy than any other state of lower spin arising from the same configuration, was deduced from atomic spectra immediately before the advent of modern quantum mechanics. Since then, the lower energy of the state of higher multiplicity has generally been explained by assuming greater electron repulsion in the lower spin states. Numerous calculations^{1–13} have shown, however, that the traditional explanation of Hund's rule is invalid. In every neutral species for which the requisite calculations have been carried out, the electron–electron repulsion, V_{ee} , is greater in the high spin state, and the lower energy of the high spin state is due to its greater electron–nucleus attraction, V_{en} . I show here that Hund's rule is a consequence of the Fermi correlation between electrons with parallel spin which leads to less screening of the nucleus in the high spin state.

Consider the singlet and triplet states arising from the $1s2s$ configuration of helium. In accordance with Hund's rule, the triplet has the lower energy but contrary to the traditional interpretation of Hund's rule, V_{ee} is greater in the triplet state.^{4,5} According to the virial theorem the total energy is equal to one-half the potential energy expectation value, which is simply the sum of V_{en} and V_{ee} . Thus, the larger V_{ee} and lower energy of the triplet state implies that V_{en} must be greater in the triplet state. This in turn implies a larger effective nuclear charge, Z_{eff} , or equivalently each electron experiences less screening of the nucleus in the high spin state. Previous calculations¹⁴ have shown that there is greater screening in the singlet state. The radial densities in the He isoelectronic sequence can be effectively superimposed¹⁴ by plotting $D(r_1)/(Z-\delta)$ against $(Z-\delta)r_1$, where Z is the atomic number and δ is an adjustable parameter. The 2^3S curves can be brought into close coincidence with $\delta=0.59$, while superposition of the 2^1S curves is best achieved with $\delta=0.80$. The electron clouds become more compact as Z increases and, therefore, it is natural to interpret the quantity $(Z-\delta)$, which brings about the superposition, as the

effective nuclear charge and δ as a screening constant. The effective nuclear charges are listed in Table 1 together with the total energy, $V_{en} = -2Z\langle 1/r_1 \rangle$ and $V_{ee} = \langle 1/r_{12} \rangle$ in the 2^3S and 2^1S states of He, as calculated⁵ from good approximations to the exact wavefunctions.

The exact pair distribution function, $f(r_{12})$, shows that the probability of the two electrons coming within about 0.5 AU of each other is vanishingly small in the 2^3S state, whereas there is a significant probability of finding the two electrons close together in the 2^1S state⁵. The difference is due to the antisymmetry principle which requires the total wavefunction, including spin, to be antisymmetric with respect to interchanging the coordinates of any two electrons. Such antisymmetry implies that the wavefunction is equal to zero whenever two electrons with parallel spin are at the same point. In effect, the antisymmetry principle prevents electrons with parallel spin from com-

Table 1 Energies of the 2^3S and 2^1S states of He (AU)

Quantity	2^3S	2^1S
E_{total}	-2.1752	-2.1459
V_{ee}	0.2682	0.2495
V_{en}	-4.6186	-4.5413
Z_{eff}	1.41	1.20

Table 2 Energies of the 2^3P and 2^1P states of He (AU)

Quantity	2^3P	2^1P
E_{total}	-2.1332	-2.1238
V_{ee}	0.2666	0.2450
V_{en}	-4.5330	-4.4927*
Z_{eff}	1.13	1.04

* The value of $\langle 1/r_1 \rangle$ in ref. 2 should read 1.12317751.

Table 3 Energies of the 2^3P and 2^1P states of Ne^{8+} (AU)

Quantity	2^3P	2^1P
E_{total}	-60.3175	-60.0557
V_{ee}	2.1059	2.2927
V_{en}	-122.7408	-122.4040

ing close to one another. The Fermi correlation, due to the antisymmetry principle, only applies to electrons with parallel spin and is in addition to the Coulomb correlation which applies to every pair of electrons. The Fermi correlation leads to a Fermi hole¹⁵, a deficiency of charge of the same spin as the electron in question, in the charge cloud around a chosen electron. Thus, Fermi correlation keeps the two electrons apart in the 2^3S state¹⁵, with the result that there is less screening of the nucleus. As each electron experiences a larger Z_{eff} , there is a concomitant contraction of the electron cloud leading to a larger V_{en} . As the electron cloud becomes more compact, some of the gain in V_{en} is lost by an increase in V_{ee} . Nonetheless, the greater V_{en} in the 2^3S state more than compensates for the additional V_{ee} .

A similar situation is observed² in the $1s2p$ states of He (see Table 2) except that the extra V_{en} in the 2^3P state is less than twice the extra V_{ee} . Furthermore these states provide an interesting example of what happens as the electron cloud becomes more compact. As the atomic number, Z , increases, the difference between V_{ee} in the triplet and singlet states diminishes, until at $Z = 4$, the electron-electron repulsion is greater in the 2^1P state. All the way up to $Z = 10$, which is as far as the accurate calculations² have been carried out, V_{en} remains greater in the triplet and the singlet has a higher energy. The relevant energies are listed in Table 3 for Ne^{8+} . The dominance of the V_{en} term confirms the screening interpretation given above, while the reversal of the V_{ee} values in highly charged ions requires further explanation. The Fermi correlation¹⁵ is essentially a local effect^{10,16} which is strongest at short inter-electronic distances. The K shell part of the electron cloud in Be^{2+} , B^{3+} and so on, is more compact than in He. With one electron in a very compact shell, the Fermi correlation is only important when an electron of the same spin in the outer shell approaches the nucleus. In these conditions, the Fermi correlation leads to substantial angular correlation so that the two electrons of the triplet state will tend to be on opposite sides of the nucleus. The Fermi correlation is not present in the 2^1P and because there is less angular correlation, V_{ee} is greater in the singlet state. When the inner shell is more diffuse, Fermi correlation will lead to proportionately less angular correlation. Of course, the electrons will be kept apart and there will be less screening of the nucleus in the triplet, but now the electrons can be kept apart and yet have a greater probability of being on the same side of the nucleus, in which case, less screening leads to greater V_{en} and the concomitant contraction of the triplet electron cloud as described above. This explanation is supported by the data² for the $1s3p$, $1s4p$ and $1s5p$ states where the switch in the relative values of V_{ee} occurs at $Z = 4$, as in the $1s2p$ states. The K shells of the $1np$ states are essentially independent of n whereas the diffuseness of the L shells increases rapidly with n .

The $1P$ and $3P$ states of the $1s^22s2p$ configuration of Be, B^+ and C^{2+} follow the same pattern^{12,13}. Namely, the magnitude of V_{en} is greater in the triplet state, while V_{ee} is greater in the triplet state of Be and B^+ but not in the more compact C^{2+} ion. From the expectation values¹² of r_{11} , Z_{eff} can be shown to be larger in the triplet state in agreement with the screening explanation of Hund's rule. Less accurate calculations^{3,17} for the $3P$, $1D$ and $1S$ states of the $1s^22s^22p^2$ configuration of the C isoelectronic sequence further support my scenario. An excellent molecular example is provided by the lowest $^1\Pi_u$ and $^3\Pi_u$ states of H_2 , for which V_{ee} has been shown⁸⁻¹⁰ to be greater in the triplet. As in every case studied to date, the lower energy of the high spin state is due to its greater V_{en} .

Finally, the explanation of Hund's rule proposed here, according to which the effective nuclear charge is greater in the higher spin states due to Fermi correlation between electrons with parallel spin, is consistent with the smaller size of the high spin state of an atom¹⁸. The latter observation has been confirmed by experimental studies of scattering cross-sections¹⁹.

I discussed the central ideas of this letter with the late Professor C. A. Coulson FRS during our research on correlation holes.

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1. Messmer, R. P. & Birss, F. W. *J. phys. Chem.* **73**, 2085-2086 (1969).
2. Accad, Y., Pekeris, C. L. & Schiff, B. *Phys. Rev. A* **4**, 516-536 (1971).
3. Katriel, J. *Theor. chim. Acta* **23**, 309-315 (1972).
4. Kohl, D. A. *J. chem. Phys.* **56**, 4236-4238 (1972).
5. Boyd, R. J. & Coulson, C. A. *J. Phys.* **B6**, 782-793 (1973).
6. Colpa, J. P. & Islip, M. F. *J. Molec. Phys.* **25**, 701-705 (1973).
7. Killingbeck, J. *Molec. Phys.* **25**, 455-459 (1973).
8. Colbourn, E. A. *J. Phys.* **B6**, 2618-2624 (1973).
9. Colbourn, E. A. & Coulson, C. A. *J. Phys.* **B7**, 1574-1581 (1974).
10. Colbourn, E. A. *J. Phys.* **B8**, 1926-1933 (1975).
11. Colpa, J. P., Thakkar, A. J., Smith, V. H. Jr & Randle, P. *Molec. Phys.* **29**, 1861-1875 (1975).
12. Shim, I. & Dahl, J. P. *Theor. chim. Acta* **48**, 165-174 (1978).
13. Tatewaki, H. & Tanaka, K. *J. chem. Phys.* **60**, 601-606 (1974).
14. Boyd, R. J. *Theor. chim. Acta* **33**, 79-86 (1974).
15. Boyd, R. J. & Coulson, C. A. *J. Phys.* **B7**, 1805-1816 (1974).
16. Katriel, J. *Phys. Rev. A* **5**, 1990-1992 (1972).
17. Katriel, J. & Pauncz, R. *Adv. Quantum Chem.* **10**, 143-185 (1977).
18. Boyd, R. J. *Nature* **250**, 566-567 (1974).
19. Pichou, F., Huetz, A., Joyez, G., Landau, M. & Mazeau, J. *J. Phys.* **B9**, 933-944 (1976).

Exosat observation of the candidate X-ray counterpart of Geminga

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During a search for X-ray counterparts of the COS B ≥ 100 MeV γ -ray sources (see refs 1, 2), observations using the Einstein Observatory revealed a new X-ray source, 1E0630+178, inside the $\sim 1/2$ square degree error box of the γ -ray source 2GC195+4, 'Geminga'³. On the basis of its interesting properties, this source has been proposed as the X-ray counterpart of Geminga. A faint ($m_v \sim 21$), star-like object lying at the inner boundary of the HRI 98% error box has been suggested⁴⁻⁷ as the optical counterpart of the X-ray (and thus the γ -ray) source. No radio counterpart has been detected, despite several searches^{7,12,13}. The region of Geminga was observed with the European Space Agency (ESA) Exosat satellite on 9-10 September 1983, from 22.30 to 07.55 UT. Due to malfunctions in orbit, both Position Sensitive Detectors of the low energy (LE) telescopes were not operable, and only the Channel Multiplier Arrays (CMA1 and 2) gave useful data. The medium energy (ME) and Gas Scintillation Proportional Counter (GSPC) instruments also recorded data although their sensitivity is insufficient to detect this soft ≈ 0.1 UFU source, also not detected by Einstein's Monitor Proportional Counter (MPC).

The CMA energy response is dependent on the input filters used. For this observation, the CMA1 used an 'aluminium/parylene' (Al/Par) filter, and the CMA2 a thin (3,000 Å) Lexan one. The filter transmission curves are given in ref. 10, Fig. 3.3. Briefly, the Al/Par filter is mostly transparent between 30 and 60 eV and then again between 300 eV and 2 keV, while the Lexan filter transmits well in the interval 30-300 eV and is similar to the Al/Par above 300 eV.

Figure 1a, b shows the central region of the CMA fields. The brightest source in the fields (number 1) is coincident with E10630+178; HS1 and HS2 are instrumental artefacts known as hotspots; source number 2 is identified with star SAO095804, of m_v 8.5 and spectral type A0; and source number 3 is identified with SAO095794, $m_v = 7.2$, spectral type F8, which is actually a double star. All identifications are based on a CMA position resolution of 10-20 arc s within 30 arc s of the telescope axis. Although a good overlap exists between the regions shown in Fig. 1 (and the complete CMA fields) and the Einstein Imaging Proportional Counter (IPC) field of ref. 3, only the brightest of the Einstein sources is detected, the others being too weak

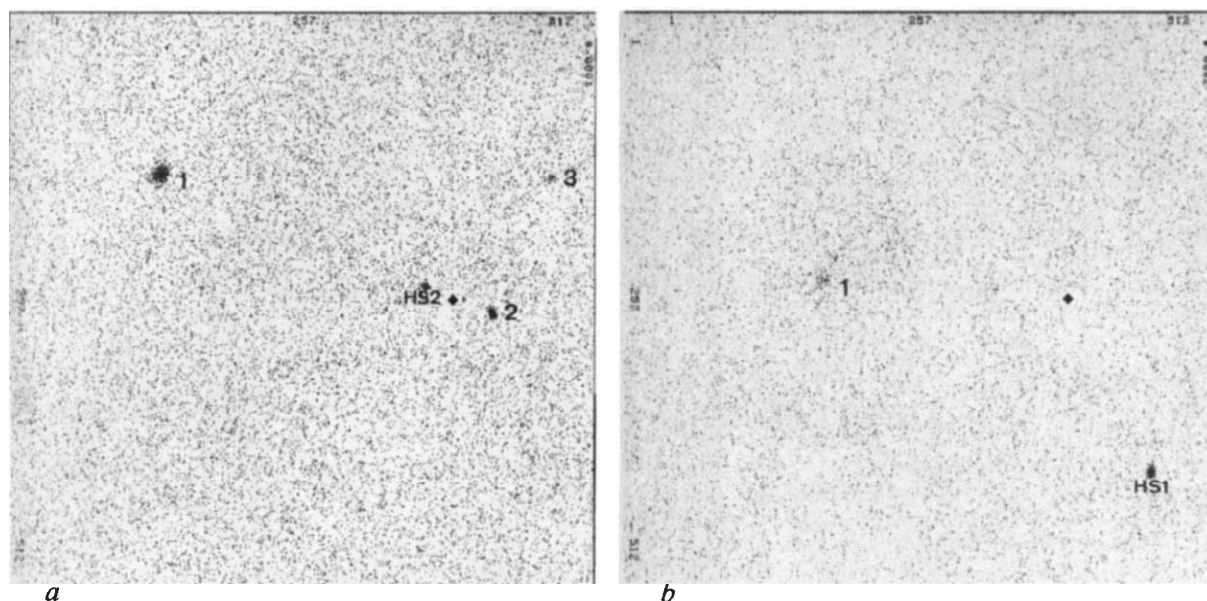


Fig. 1 The central region ($\sim 30 \times 30$ arcs) of the Exosat CMA fields, with the pointing direction ($\blacklozenge$) shown. *a*, Field with 3,000 Å Lexan filter (CMA2) exposed for 34,200 s, of which 27,500 s constituted the useful observing time. HS1 is an instrumental hotspot, and three sources are seen: number 1 with 700 counts under its PSF, number 2 with 490 counts and number 3 with 60 counts. Source number 1 coincides with 1E0630 + 78, the proposed X-ray counterpart of Geminga. *b*, As *a*, but with the Al/Par filter. Only source number 1 is now visible, with ~ 100 counts.

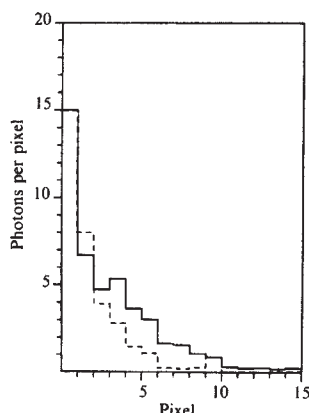


Fig. 2 Comparison of PSF for sources 1 and 2, with peak height normalized to number 1. A constant background level of 0.46 photons per pixel was subtracted. The Geminga (number 1) profile (solid line) shows wider wings, probably due to its off-axis position.

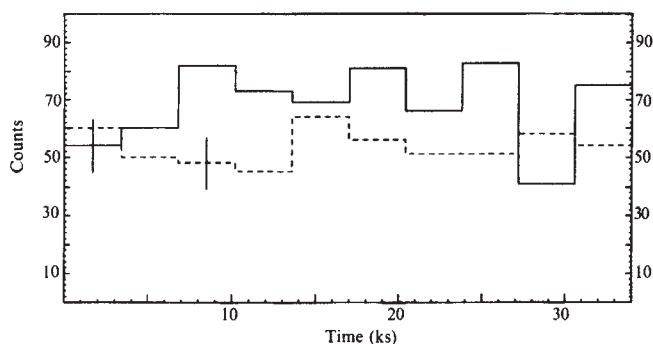


Fig. 3 Comparison of the time profiles for Geminga (solid line) and source number 2 (8th magnitude star, dashed line). Both profiles have been corrected for a variable (20%) dead time. The background, averaged from Fig. 1, has been subtracted. The start time of the observation (origin of abscissa) was 9 September 1983, at 22.30 UT.

($\sim 10^{-13}$ erg cm $^{-2}$ s $^{-1}$) for this CMA exposure. The two stars (numbers 2 and 3) are at the boundary of the IPC field and have probably been detected by the CMA2 due to its UV sensitivity. This is confirmed in the case of source number 2, where the 'sum signal' distribution shows the presence of heavy UV contamination. The radial brightness profile of sources 1 and 2 is shown in Fig. 2. Both profiles are consistent with the known CMA point spread function (PSF, as described on the Exosat calibration file on the final observation tape, FOT), taken once at about 20 arcs from the image centre (number 1) and then (number 2) near to it.

The available data on the CMA performances indicate that the broader profile of Geminga relative to source number 2 is fully accounted for by its off-axis position, and thus no evidence of extension is seen. The preliminary position of number 1 ($\alpha = 6$ h 30 min 58.2 s, $\delta = 17^\circ 48' 36''$) contains in its estimated error radius the more accurate High Resolution Imager (HRI) box given in ref. 3. No fine correction for spacecraft attitude ('deblurring') has yet been introduced.

The CMA detectors cannot provide spectral data apart from those related to the filter transmissions. In the case of Geminga, the limited source flux meant that only two filters could be used

in the 10-h observation. With only two broad energy windows, no spectral fitting was attempted. The Exosat data are simply consistent with the Einstein finding of a very soft spectrum (for example, energy index $\alpha \sim -2.3$), with a very low N_H (10^{19} – 10^{20} cm $^{-2}$) for its galactic latitude of $+4^\circ$.

In this preliminary analysis the source flux has been estimated to be consistent with the one given for the IPC range in ref. 3 (2.2×10^{-12} erg cm $^{-2}$ s $^{-1}$).

The main new result given by the Exosat observation refers to a time profile of the source obtained with virtually continuous coverage during the 10-h observation.

Although source number 1 was detected by both CMA instruments, the counting statistics of the CMA1 image (Fig. 1*a*) are too poor for a useful time analysis. On the other hand, the different configurations of the two CMA instruments (the very different dead time corrections) prevent an easy summation of the two images. Therefore, the CMA2 field alone was used in the time analysis. The continuous elapsed observation time was $\sim 34,200$ s, with a 20% dead time correction, due to the inability of the CMA detector to record every X-ray photon, especially during the irregular increases in the background rate that were probably related to solar activity.

Figure 3 (solid line) shows the source time profile after subtraction of the measured background, where all the source counts within 10 pixels from the centroid are taken (~ 570) to maximize the signal-to-noise ratio; for comparison, the broken line shows the same profile for source number 2 (star), which has ~ 450 counts, with the background also subtracted. The profiles have been corrected for the appropriate dead time value per bin. The error bars shown are for a Poisson distribution and include errors for the background. A standard χ^2 test was performed against the hypothesis of a constant source for both profiles: for Geminga this yielded $\chi^2 = 16.25$ (9 d.f.), or a 6% probability that the observed profile is due to a constant source. For the star, the $\chi^2 = 6.37$ (9 d.f.). A Kolmogorov-Smirnov test was also performed, and the outcome was compatible. This result, although suggestive, does not provide sufficient evidence for source variability. Obviously, a longer observation is needed to assess its statistical significance.

In view of the above data, we note that Hertz and Grindlay⁹ have reported a possible variation (at the $\sim 2\sigma$ level) of the Einstein source in the IPC data, on a time scale of a few thousand seconds⁸—a large fraction of the whole observation. Mereghetti¹¹, using all the available reprocessed Einstein data, has found a similar result using a different technique for analysis.

As to the nature of the emitting object, the result presented here does not alter the conclusions reached earlier (see refs 3, 7),

linking the observed γ -X-optical emissions, assumed related, to a compact object, such as a neutron star. Thus an accurate check on all the available data for other time variations and/or periodicities becomes important. This is underway on the five X-ray data sets available (three Einstein and two Exosat) and will be performed on optical observations made in January 1984 at the Canadian French Hawaiian Telescope. Further optical observations, including fast photometry, would be highly desirable, as would further ultrahigh energy γ -ray data ($\geq 10^{12}$ EV), where interesting results have been obtained (ref. 14) and more data are being collected (T. Weekes, personal communication).

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1. Caraveo, P. A. *Space Sci. Rev.* **36**, 207 (1983).
2. Bignami, G. F. & Hermesen, W. A. *Rev. Astr. Astrophys.* **21**, 67 (1983).
3. Bignami, G. F., Caraveo, P. A. & Lamb, R. C. *Astrophys. J. Lett.* **272**, L9 (1983).
4. Caraveo, P. A., Bignami, G. F., Vigroux, L. & Paul, J. A. *Astrophys. J. Lett.* **276**, L45 (1984).
5. Halpern, J. P., Grindlay, J. E., Bignami, G. F. & Caraveo, P. A. *IAU Circ. No.* 3907 (1984).
6. Halpern, J. P. & Grindlay, J. E. *Bull. Am. astr. Soc.* **15**, 909 (1983).
7. Caraveo, P. A., Bignami, G. F., Vigroux, L., Paul, J. A. & Lamb, R. C. *Adv. Space Res.* (1984b).
8. Hertz, P. thesis, Harvard Univ. (1983).
9. Herz, P. & Grindlay, J. E. *Astrophys. J.* (in the press).
10. Exosat Observers Guide Rev. 1. (ESA, January 1984).
11. Mereghetti, A. thesis, Univ. Milan (1984).
12. Sieber, W. & Schlickeiser, R. *Astr. Astrophys.* **113**, 314 (1982).
13. Spoelstra, T. A. Th. & Hermesen, W. *Astr. Astrophys.* (in the press).
14. Zyskin, Yu. & Mukanov, D. *Soviet Astr. Lett.* **9**, 117 (1983).

Response of two atmospheric general circulation models to sea-surface temperature anomalies in the tropical East and West Pacific

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A substantial anomalous warming of sea-surface temperatures (SSTs) in the tropical Pacific Ocean (an 'El Niño') can have profound effects on the atmospheric mid-latitude long waves, leading to severe weather anomalies¹⁻⁵. However, the magnitude and position of these weather anomalies seem to vary considerably between winters in which El Niño events are in progress^{2,6,7}. In order to forecast with confidence the effects of future El Niño events, it is important to understand the physical reasons for these differences. Motivated by this, we present several atmospheric general circulation experiments which model the atmospheric response to SST anomalies in the tropical East and West Pacific. We have found that the extratropical response to a relatively small West Pacific anomaly can be stronger than and qualitatively different from the response to a much larger East Pacific anomaly. These experiments suggest a possible explanation for the difference in mid-latitude response during the 1976-77 El Niño winter and the El Niño winters of 1972-73 and 1982-83.

The first set of experiments were integrations of the global 11-layer atmospheric general circulation model (AGCM) of the UK Meteorological Office, with $2^\circ \times 3^\circ$ horizontal resolution. The model has a penetrative convection scheme and, in the version used here, non-interactive radiation with fixed zonally symmetrical cloud amounts (see refs 8, 9 for details). All experiments ran for 150 days from 28 December 1972, with fixed January radiation constants. The control integration had (fixed) January climatological SSTs. The SST anomalies added for the two experiments are illustrated in Fig. 1 (the anomaly field in Fig. 1a is taken from data for December 1972).

Figure 2 illustrates the 500-mbar streamfunction anomaly (that is, difference from control) associated with the two experiments, averaged over 150 days. Figure 2a shows a high anomaly centre at about 10°N 130°W to the north-west of the maximum SST anomaly. A low anomaly centre lies further north over the

Gulf of Alaska. The gradient between these centres gives rise to an average westerly wind anomaly of $\sim 3\text{ m s}^{-1}$. A second weak low anomaly centre lies over the southern states of the USA and over the north-east Atlantic, and a high anomaly centre over the South China Sea. The 500-mbar streamfunction anomaly pattern shown in Fig. 2b is stronger and quite different from that in Fig. 2a. There is a low centre at 15°N 140°E , to the north of the maximum in the SST anomaly, with a strong downstream response giving anomaly centres just west of the dateline, to the south of the Alaska Peninsula, over Hudson Bay and over the Gulf of Mexico. A weaker centre occurs further downstream to the west of the British Isles. The wind anomalies, up to 12 m s^{-1} over the Pacific, are stronger than and in a different position from those in expt 1, with south to southwesterlies over most of the west coast and north to northeasterlies over most of the east coast of North America. This pattern was apparent in each 30-day mean field comprising Fig. 2b.

We note that a rainfall anomaly maximum of 12 mm per day occurred at 7°S 135°W in expt 1 and at 7°N 170°E in expt 2. Although the convective rain routine used in the model tends to produce excessive rainfall rates over the tropical oceans, these values indicate that the stronger atmospheric response to the

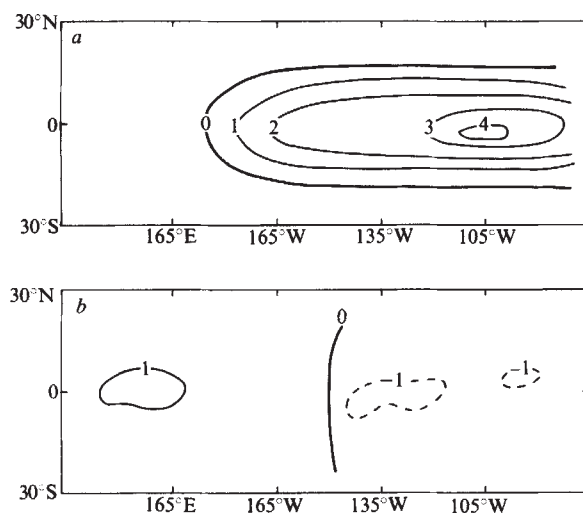


Fig. 1 SST anomaly (K). a, Expt 1; b, expt 2 (having a maximum value of 1.3 K in the West Pacific).

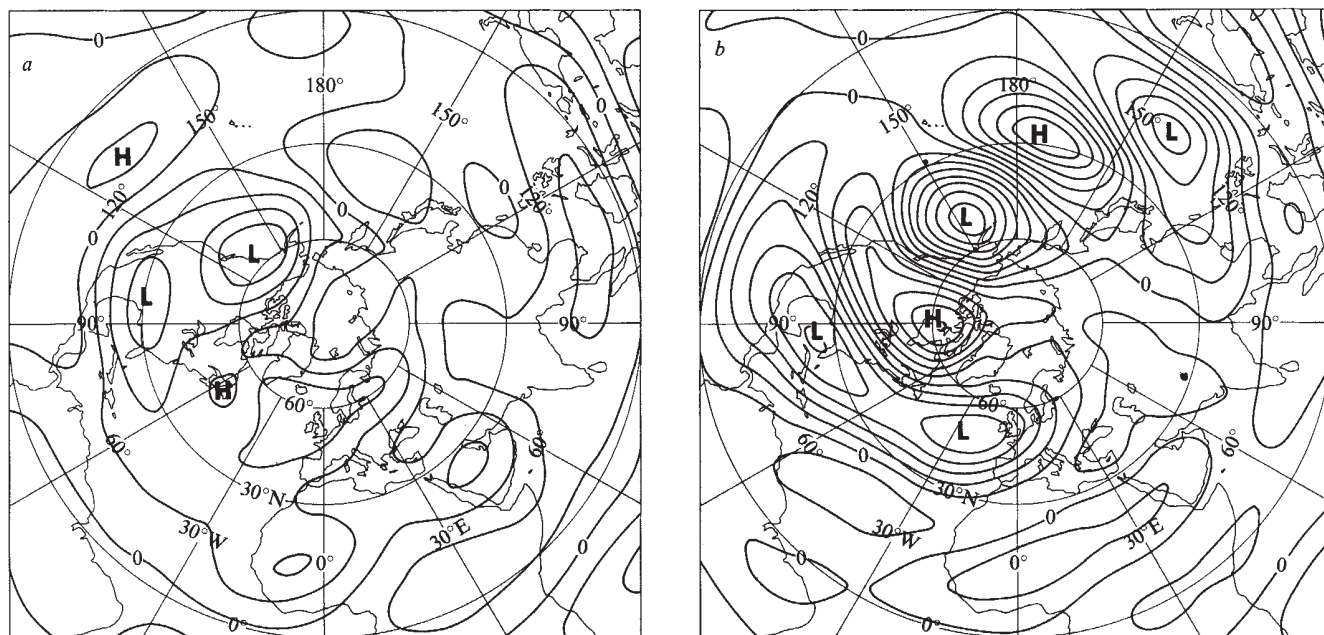


Fig. 2 500-mbar streamfunction anomaly averaged over days 1–150. *a*, Expt 1; *b*, expt 2. Contour interval is $2 \times 10^6 \text{ m}^2 \text{ s}^{-1}$.

second SST anomaly was apparently not associated with larger tropical rainfall anomalies. On the other hand, rainfall and associated divergence field anomalies extended to 15° N for the West Pacific anomaly experiment, but to only 5° N for the East Pacific anomaly experiment. Simmons¹⁰ finds 15° N to be an optimum latitude and the West Pacific an optimum longitudinal region for forcing the extratropics by tropical heating. For both experiments the tropical wind anomalies are strongly baroclinic and broadly consistent with Gill's¹¹ linear model. (The 500-mbar wind anomalies over the East Pacific in Fig. 2*a* correspond to Gill's upper-level solution, whereas the 500-mbar wind anomalies over the West Pacific in Fig. 2*b* correspond to Gill's lower-level solution. This difference is possibly due to the variation of the depth of convection over the East and West Pacific.)

In interpreting the anomaly maps of Fig. 2 we note that the control integration has a zonally asymmetrical flow, driven in part by the imposed zonal gradient in the climatological SSTs. Simmons¹⁰ has shown that many aspects of the mid-latitude winter flow can be reproduced using purely tropical forcing. The addition of the anomaly field illustrated in Fig. 1*a* considerably weakens the normal (east–west) gradient, thereby making the isotherms of the SST more zonal. As expected, therefore, the mid-latitude Pacific jet responds to this change in SST by becoming more zonal: the anomaly pattern in Fig. 2*a* corresponds to an increase in the strength of the jet over the East Pacific and a slight decrease in strength over the West Pacific. In contrast, the SST anomaly shown in Fig. 1*b* enhances the zonal tropical SST gradient across the Pacific. The response shown in Fig. 2*b* is qualitatively consistent with a downstream-propagating wave-train¹² forced by the positive SST anomaly in the tropical West Pacific. The response is also remarkably similar to the stationary mode described by Simmons *et al.*¹³ (see their Fig. 14*b*), suggesting that the pattern in Fig. 2*b* may be related to a weakly unstable barotropic mode in the AGCM.

The atmospheric height anomaly patterns over the East Pacific and North America for January 1973⁷ and 1983² bear some resemblance to the model response in Fig. 2*a*, as they include a high anomaly centred over the East Pacific at about 20° N , a low anomaly directly to the north and relatively weak anomalies (and mild conditions) over the USA. The observed pattern for

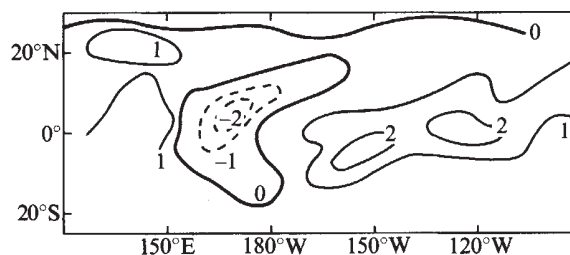


Fig. 3 SST anomalies (K) for January 1977 (UK Meteorological Office SST archives).

January 1977⁶ is quite different, with strong anomalies over the USA (see Fig. 4*d*) leading to an exceptionally severe winter over much of the continent. Counterparts to the pattern in Fig. 4*d* can be found in the model response (Fig. 2*b*) to the SST anomaly pattern in Fig. 1*b*. Although the real SST pattern for January 1977 (Fig. 3) is quite different in the East Pacific from that used in the model, we note that there was a warm anomaly of up to 1.5 K in the West Pacific centred at 140° E .

To test further the suggestion that the atmospheric anomaly pattern in the winter of 1976–77 may have been largely due to this warm West Pacific SST anomaly, a set of 50-day forecast experiments was run on a hemispheric version of the UK Meteorological Office 5-level AGCM¹⁴. These integrations used initial conditions for 14 December 1976 and were run (1) with climatological SSTs (control), (2) with the full tropical Pacific anomaly as in Fig. 3, (3) with only the warm anomaly west of 155° E and (4) with only the anomaly east of 155° E . Figure 4*a–c* shows the 500-mbar streamfunction response (difference from control), averaged over days 21–50, for the full SST anomaly, the West Pacific anomaly and the East Pacific anomaly. The observed 500-mbar geostrophic streamfunction anomaly for January 1977 is shown in Fig. 4*d*. Over the Pacific and North America the model response to the full and West Pacific anomalies is very similar (Fig. 4*a, b*) and there is a correspondence with the pattern shown in Fig. 2*b* (although a phase shift

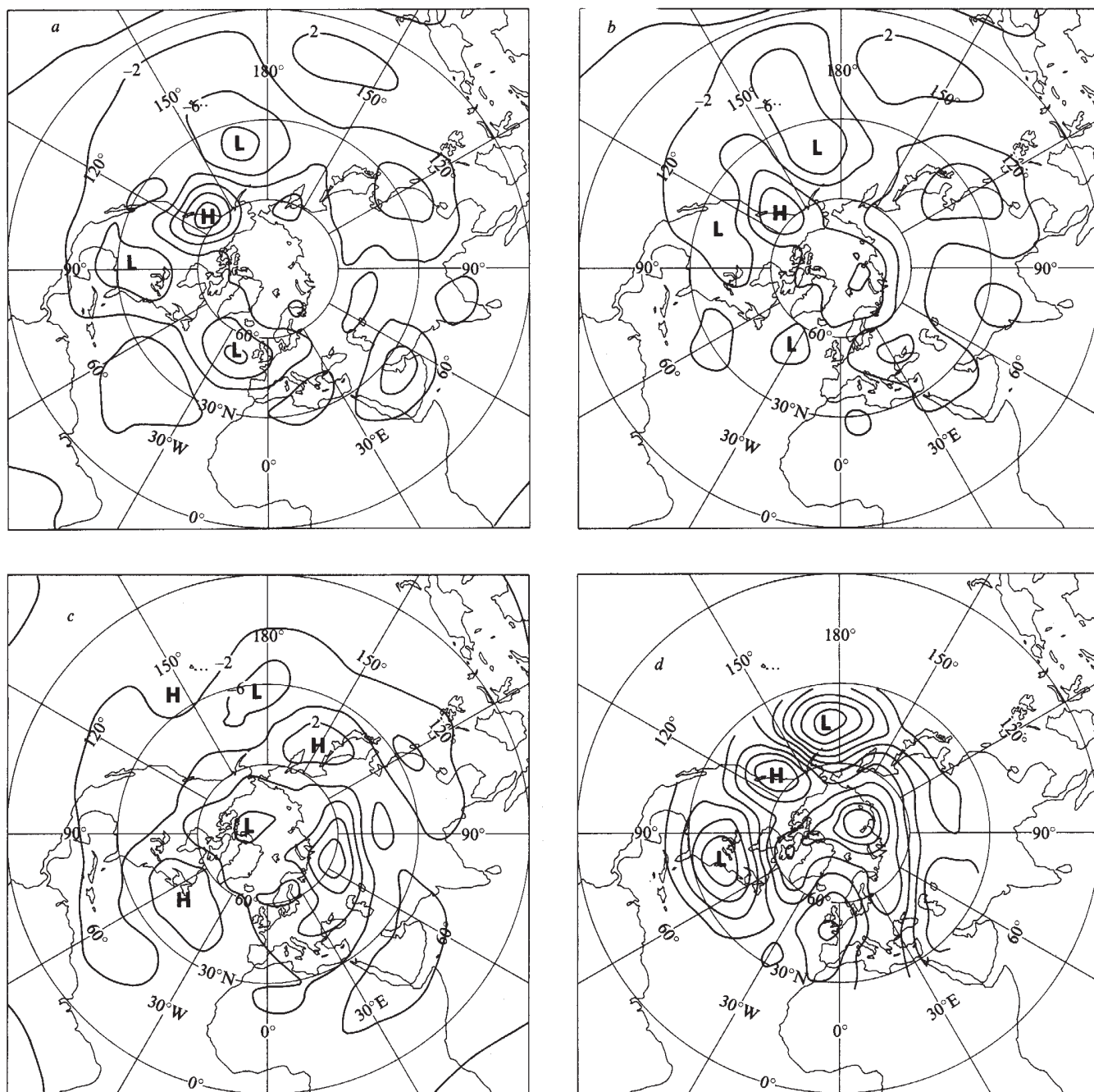


Fig. 4 500-mbar streamfunction anomaly for days 21–50 of the forecast experiment begun on 14 December 1976. *a*, With SST anomalies taken from Fig. 3 for January 1977; *b*, with SST anomalies taken from Fig. 3 west of 155° E only; *c*, with SST anomalies taken from Fig. 3 east of 155° E only; *d*, observed 500-mbar geostrophic streamfunction anomaly for January 1977. Contour interval is $4 \times 10^6 \text{ m}^2 \text{ s}^{-1}$ in each case.

between the 5- and 11-level model responses is apparent, possibly due to a tendency in the 11-level model to develop excessively strong zonally-averaged mid-latitude westerlies). The low centre over the North Pacific and the high over the west coast of North America coincide very closely with the real anomalies. When the West Pacific SST anomaly is removed the response over the Pacific and North America is much weaker (see Fig. 4c).

In conclusion, results from both AGCMs suggest that the extratropical response to relatively small SST anomalies in the warm tropical West Pacific can be large and may explain the differences in the atmospheric response to past El Niño events. Although, of course, the preliminary results presented here are not conclusive, they do highlight the importance of extensive AGCM modelling as a means of understanding the crucial role

of the influence of the tropical Pacific Ocean on global atmospheric circulation patterns.

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1. Blackmon, M. L., Geisler, J. E. & Pitcher, E. J. *J. Atmos. Sci.* **40**, 1410–1425 (1983).
2. Quiroz, R. S. *Mon. Weath. Rev.* **111**, 1685–1706 (1983).
3. Rowntree, P. R. *Q. Jl R. met. Soc.* **98**, 290–321 (1972).
4. Van Loon, H. & Madden, R. A. *Mon. Weath. Rev.* **109**, 1150–1162 (1981).
5. Van Loon, H. & Madden, R. A. *Mon. Weath. Rev.* **109**, 1163–1168 (1981).
6. Wagner, J. A. *Mon. Weath. Rev.* **105**, 553–560 (1977).
7. Wagner, J. A. *Mon. Weath. Rev.* **101**, 381–386 (1973).
8. Saker, N. J. *Met. Off. 20 tech. Note 11/30* (UK Met. Office, Bracknell, 1975).
9. Lyne, W. H. & Rowntree, P. R. *Met. Off. tech. Note 11/70* (UK Met. Office, Bracknell, 1976).
10. Simmons, A. J. *Q. Jl R. met. Soc.* **108**, 503–534 (1982).
11. Gill, A. R. *Q. Jl R. met. Soc.* **106**, 447–462 (1980).
12. Hoskins, B. J. & Karoly, D. J. *J. Atmos. Sci.* **38**, 1179–1196 (1981).
13. Simmons, A. J., Wallace, J. M. & Branstator, G. *J. Atmos. Sci.* **40**, 1363–1392 (1983).
14. Corby, G. A., Gilchrist, A. & Rowntree, P. R. *Meth. comput. Phys.* **17**, 67–110 (1977).

Excess ^{227}Ac in deep ocean water

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Understanding the nature and rates of processes by which materials are transported and mixed in the deep ocean is essential for evaluating environmental effects and risks involved in human uses of the ocean, such as mining of manganese nodules and deep ocean dumping of wastes. I report here the first measurements of ^{227}Ac (half life, $t_{1/2}$, 21.8 yr) in seawater. The results clearly show the presence of excess ^{227}Ac relative to its progenitor ^{231}Pa (see Fig. 1) in deep waters below 3 km, suggesting that the ^{227}Ac is supplied by diffusion from sediments. Two other natural radionuclides, ^{222}Rn (ref. 1) and ^{228}Ra (ref. 2) which have bottom sources, have proved to be useful for determining rates of mixing from days to decades. The excess ^{227}Ac can be used as a novel tracer for basin-wide circulation and mixing on time scales up to 100 yr.

In January to March 1982, the Cephæus expedition (KH-82-1), using RV *Hakuho-Maru*, occupied three large-volume stations in the western North Pacific: Ce-5 ($25^{\circ}00' \text{N}$, $169^{\circ}59' \text{E}$), Ce-8 ($12^{\circ}45' \text{N}$, $173^{\circ}14' \text{E}$) and Ce-13 ($12^{\circ}00' \text{N}$, $152^{\circ}30' \text{E}$). The vertical profiles of ^{227}Ac and ^{231}Pa were measured at the latter two subtropical stations. (The hydrographic data are described in ref. 3.) The detailed analytical procedure for the various radionuclides found in a 230-l aliquot of seawater collected in a PVC large volume sampler will be given elsewhere. In brief, ^{227}Ac and ^{231}Pa , together with other actinides, were co-precipitated with iron hydroxide and removed from the water. ^{227}Ac was then separated from Th, Pa and other actinides by ion exchange. Attempts to measure by α -spectrometry the *in situ* activity of ^{227}Th which should have been in equilibrium with the parent ^{227}Ac in all the water except at the surface^{4,5} were not entirely successful because of interference by the daughter nuclides of ^{228}Th . The method can only be used for deep-water samples with relatively high activity ratios of $^{227}\text{Th}/^{228}\text{Th}$ and often involves large uncertainties (see Fig. 2). Therefore, the ^{227}Ac fraction was stored for more than 4 months during which its daughter ^{227}Th ($t_{1/2}$, 18.7 days) increased to be in almost secular equilibrium concentration with ^{227}Ac . The daughter ^{227}Th was determined using a known amount of ^{230}Th as a yield tracer, and the ^{227}Ac concentration was calculated from its activity. It was assumed that ^{227}Ac was quantitatively co-precipitated with iron hydroxide. The laboratory experiment, using a ^{227}Ac tracer and a 20-l aliquot of open ocean surface water, showed that the assumption was valid within a 3% error. Any incomplete co-precipitation of ^{227}Ac , if it occurs in expanded volumes, makes

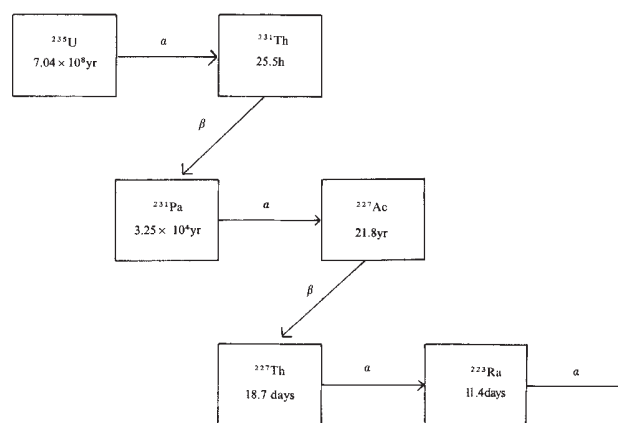


Fig. 1 A portion of ^{235}U decay chain.

the real values higher than those given in Table 1. The loss of iron hydroxide precipitate during handling and processing was corrected for following the recovery of iron ($\sim 80\%$). That the loss of ^{227}Ac during the ion exchange procedure is negligible⁶, is also confirmed by agreement, within experimental errors, between *in situ* ^{227}Th results and the ^{227}Ac , determined as above, for some deep-water samples. For ^{231}Pa , a radiochemical method was used, including purification by ion exchange and solvent extraction procedures, and α - and β -spectroscopy using ^{233}Pa ($t_{1/2}$, 27 days) to determine² the overall yield.

The results for ^{227}Ac are given in Table 1 with $\pm 1\sigma$ counting errors. The procedural blank was not corrected, so that some surface values could be due to the blank, although this value is not significant for deep samples. The data for ^{231}Pa and their geochemical implications will be given elsewhere but the vertical feature based on the two profiles is shown in Fig. 2. Because radioactive equilibrium with ^{227}Ac is expected, the values of *in situ* ^{227}Th activity at Ce-5, and those measured by the moored MnO_2 -fibre technique at 30°N , 146°E reported earlier⁴ are also plotted in Fig. 2. Both ^{227}Ac and ^{231}Pa increase from values close to the detection limit in surface waters, to $0.7 \times 10^{-3} \text{ d.p.m. kg}^{-1}$ at a depth of around 3 km, with no significant departure from their equilibrium relationship. Below this depth, however, a marked increase of ^{227}Ac in contrast to a slight decrease of ^{231}Pa with depth was observed. The existence of excess ^{227}Ac compared with ^{231}Pa implies that there is a source of ^{227}Ac in the bottom waters other than production from the parent ^{231}Pa . The trend that the excess ^{227}Ac increases towards the sea floor strongly suggests that the ^{227}Ac is supplied from the underlying sediment in an analogous way to the Ra isotopes. But it is unclear what geochemical similarities there are between Ac and Ra, because little is known about the chemistry of Ac in natural aquatic environments. Laboratory studies suggest that chemical properties of actinium compounds resemble those of lanthanum but that the hydroxide is more basic (and hence more soluble in seawater) than lanthanum hydroxide⁷. Recently, Klinkhammer *et al.*⁸ showed the nutrient-like depth profiles of charge 3+ rare earth elements, such as Si, Ba and ^{226}Ra , which are indicative of a deep regeneration cycle. Considering these, it seems likely that Ac is, diagenetically and/or radiogenetically (due to α -recoil effect), released from sediments to the overlying water.

More insight into the processes controlling ^{227}Ac come from comparing its oceanic distribution with that of ^{228}Ra . Although ^{228}Ra has not yet been measured, the general vertical feature may be deduced from its daughter ^{228}Th ($t_{1/2}$, 1.9 yr). The ^{228}Th profiles [ref. 4, and unpublished data] show an increase towards the bottom which is similar to the case of excess ^{227}Ac (Fig. 3). A strong positive correlation exists between ^{228}Th and excess ^{227}Ac (or ^{227}Th) with activity ratios of around unity. The bottom standing crop of excess ^{227}Ac ($\sim 0.3 \text{ d.p.m. cm}^{-2}$) is nearly the same at that of ^{228}Th . This produces an ^{227}Ac flux of

Table 1 Concentration of ^{227}Ac in seawater

Depth (m)	^{227}Ac ($\times 10^3 \text{ d.p.m. kg}^{-1}$)	Depth (m)	^{227}Ac ($\times 10^3 \text{ d.p.m. kg}^{-1}$)
Ce-8, 5727 m		Ce-13, 5,925 m	
10	0.05 ± 0.02	10	0.08 ± 0.03
127	0.06 ± 0.02	98	0.06 ± 0.02
196	0.07 ± 0.02	147	0.08 ± 0.03
296	0.17 ± 0.05	197	0.14 ± 0.03
394	0.13 ± 0.03	300	0.02 ± 0.02
504	0.28 ± 0.06	394	0.12 ± 0.03
605	0.26 ± 0.04	500	0.21 ± 0.03
803	0.41 ± 0.06	692	0.29 ± 0.05
988	0.36 ± 0.08	980	0.38 ± 0.06
1,495	0.58 ± 0.08	1,494	0.64 ± 0.09
1,985	0.71 ± 0.08	2,493	0.56 ± 0.09
2,980	0.83 ± 0.10	3,482	0.75 ± 0.10
3,997	1.58 ± 0.13	4,505	1.45 ± 0.15
5,377	1.96 ± 0.19	5,589	2.26 ± 0.23
5,676	2.09 ± 0.18	5,870	2.68 ± 0.25

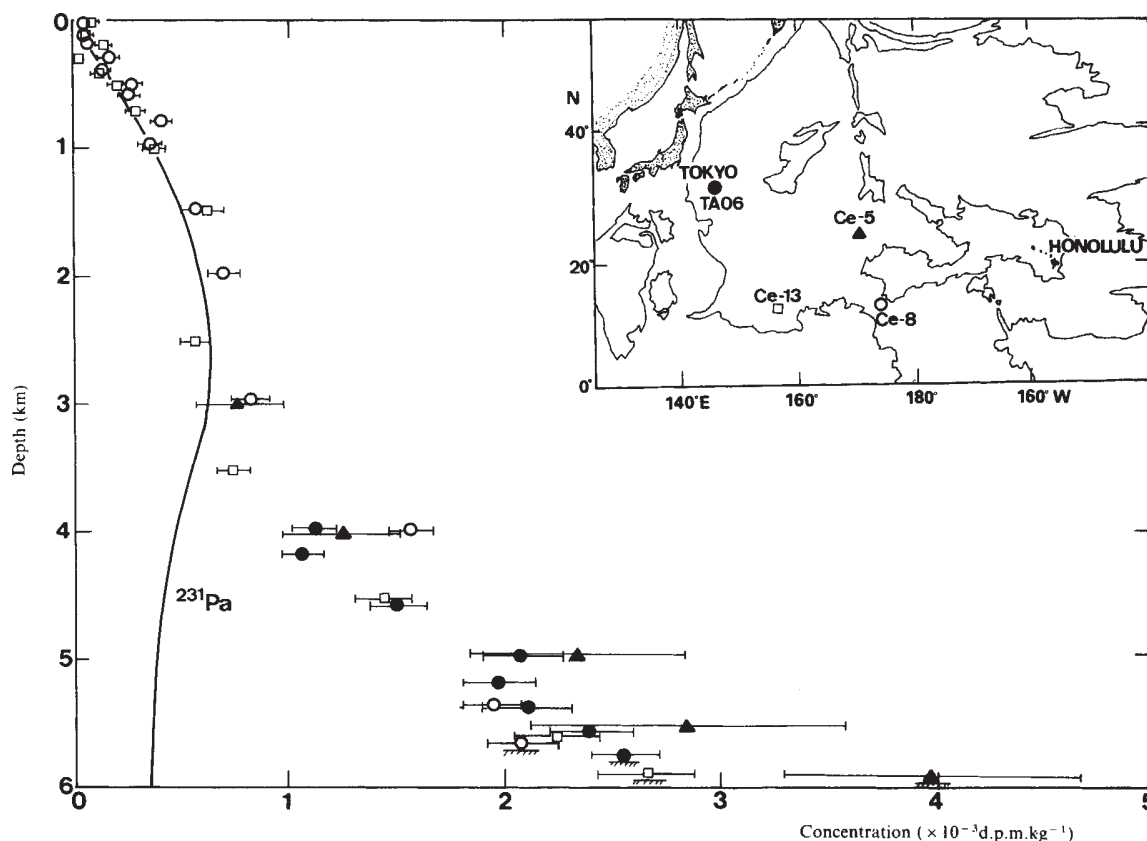


Fig. 2 Depth distributions of ^{227}Ac (○, Ce-8; □, Ce-18) in the western North Pacific. ▲, The *in situ* activity of ^{227}Th at Ce-5; ●, ^{227}Th data reported earlier⁴. The ^{231}Pa profile is based on the data at Ce-8 and Ce-13. The counting error for ^{231}Pa measurements was ~15% at a concentration of 0.5×10^{-3} d.p.m. kg^{-1} . The inset map shows station locations.

$0.01 \text{ d.p.m. cm}^{-2} \text{ yr}^{-1}$ across the sediment–water interface corresponding to only 0.5% of the production rate from ^{231}Pa in the sediment column. This production rate is estimated by assuming that ^{231}Pa is quantitatively removed from the 5,800-m water column to pass into the underlying sediment.

The ^{227}Ac and ^{228}Ra in the bottom waters are expected to be supplied only from superficial sediments (presumably <10 cm thick) due to their relatively short half lives. Cochran⁹, using porewater measurements, has shown this for ^{228}Ra . Therefore, the fluxes of these nuclides is most likely to depend on the contents of their parent ^{231}Pa and ^{232}Th in the surface sediments, and be independent of the difference in their sediment profiles. Interestingly, measurements of Th and Pa isotopes in the top few centimetre sediments from two pilot cores obtained in this region gave a mean value of 1 ± 0.2 for the activity ratio of $^{231}\text{Pa}/^{232}\text{Th}$. This implies that the production rate of ^{228}Ra is about four times greater than that of ^{227}Ac . Because the standing crop of ^{227}Ac in the bottom water is approximately equal to that of ^{228}Ra (as deduced from ^{228}Th), the flux of ^{228}Ra out of the sediments must also be about four times that of ^{227}Ac . This suggests that the mobility of Ac in sediment is about the same as that of Ra isotopes in the western North Pacific region.

Another point that can be noted from Fig. 2 is that there is little excess ^{227}Ac in surface waters at these subtropical stations. This is in contrast to the observation that there is significant excess ^{228}Ra in open ocean surface waters, although the amount is smaller in tropical regions than in mid gyres^{10,11}. The difference can be ascribed to their fluxes from shallow water sediments. The ^{231}Pa content in shallow water sediments is considerably lower than that in deep-sea surface sediments to which large amounts of radiogenetically-produced ^{231}Pa are added from the water column, whereas the variation of non-radiogenic ^{232}Th content is small irrespective of the depth. Hence, the ^{227}Ac supplied by lateral transport from continental margins to open ocean surface water must be much smaller than that of ^{228}Ra .

Before applying a mixing model to the ^{227}Ac data for estimating the vertical eddy diffusion coefficient, the effect of scavenging on the vertical distribution needs to be evaluated. At present, the only available data for ^{227}Ac in marine particulate matter are those for sediment trap samples determined by Anderson and Fleer⁶. Their data indicate that the activity ratio of $^{227}\text{Ac}/^{230}\text{Th}$ for particles (~ 0.015 for depths >4 km) is two orders of magnitude smaller than that for seawaters obtained here (see also refs 4, 12). Thus, the box-model type scavenging residence time of Ac is estimated to be $\sim 3,000$ yr, 100 times longer than that of ^{230}Th (ref. 12). As the mean life of ^{227}Ac is only 30 yr, the scavenging can hardly influence the vertical profile of ^{227}Ac . The fluid processes by which ^{227}Ac is transported to the deep ocean interior, and the decay, are the main factors governing the distribution.

I have attempted to estimate the apparent vertical eddy diffusion coefficient (D) based on the ^{227}Ac data and a steady-state vertical mixing model given by

$$\frac{\partial}{\partial z} \left(D \frac{\partial A}{\partial z} \right) = \lambda A$$

where A is the activity of excess ^{227}Ac relative to ^{231}Pa , λ is the decay constant and z is the distance above the bottom. The boundary conditions that $A = A_0$ at $z = 0$ and $A = 0$ at $z \geq 5,000$ m are used throughout this calculation. If D is assumed to be constant with depth, then the relationship derived is $D = z_{1/2}^2 / 0.693 t_{1/2}$, where $z_{1/2}$ is the distance for $A = A_0/2$ and $t_{1/2}$ is the half life of ^{227}Ac (21.8 yr). Taking $z_{1/2} = 1,000$ m (Fig. 3), $D = 20 \text{ cm}^2 \text{ s}^{-1}$ is obtained. This value is consistent with, but at the lower end of, the range of estimates from the bottom ^{228}Th (^{228}Ra) profiles (ref. 4).

In reality, however, the apparent vertical eddy diffusion coefficient is more likely to be dependent on depth. For example, various values of D have been estimated as $\sim 50 \text{ cm}^2 \text{ s}^{-1}$ from

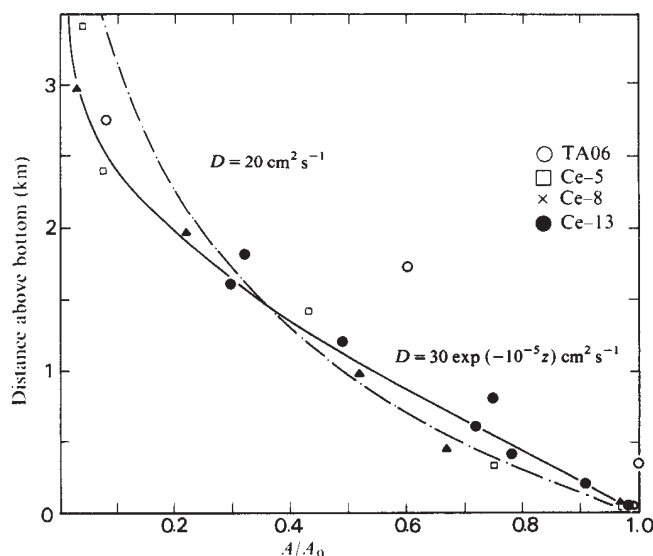


Fig. 3 The vertical profiles of bottom excess ^{227}Ac (or ^{227}Th). The values were calculated by subtracting ^{231}Pa activity measured, or if not measured, assumed, based on the profile shown in Fig. 2 from ^{227}Ac (or ^{227}Th) activity, and represented as the fraction of the extrapolated activity at the sea floor. The same symbols are used as for the stations in Fig. 2. Two model curves (see text) are also shown.

near bottom ^{222}Rn profiles, $\sim 30 \text{ cm}^2 \text{ s}^{-1}$ from ^{228}Th (^{228}Ra) profiles for the bottom 2 km, and $\sim 1 \text{ cm}^2 \text{ s}^{-1}$ from ^{14}C between 1 and 4 km in the North Pacific. Sarmiento *et al.*¹³ have demonstrated that the apparent diffusion coefficient is inversely correlated to the vertical density gradient. For waters below 3 km in the western North Pacific, the vertical gradient of potential density, although small, increases with the distance from the bottom. Considering these, as exponentially decreasing formula, $D = D_0 \exp(-\beta z)$ was assumed for the vertical variation. Then the solution of the model is given by

$$A = A_0 \exp\left(\frac{\beta z}{2}\right) \left[K_1\left(\frac{2}{\beta} \sqrt{\frac{\lambda}{D_0}} \cdot \exp\left(\frac{\beta z}{2}\right)\right) / K_1\left(\frac{2}{\beta} \sqrt{\frac{\lambda}{D_0}}\right) \right]$$

where K_1 is the modified Bessel function of the second kind of first order. Figure 3 shows the best fit curve where $D = 30 \exp(-10^{-5} z)$ (z in cm). The curve appears to be a better fit than when D is constant and also to be more consistent with the trend of variation of D estimated from other tracer distributions such as ^{222}Rn , ^{228}Ra and ^{14}C .

Despite the consistency of the vertical model, the effect of horizontal transport was implicitly ignored in the calculation. However, some oceanographers^{14,15} believe that the lateral mixing coupled with slope boundary turbulent diffusion is an important control on the distribution of chemical properties in the deep ocean. Indeed, the similarity of the vertical profiles of ^{227}Ac , regardless of the difference of locations (Fig. 2), is not inconsistent with the idea. For more detailed arguments a three-dimensional data set, as well as information on the geographical variations of ^{227}Ac flux from sediments, are needed. In any case, simultaneous use of ^{227}Ac and ^{228}Ra as tracers will provide one of the most useful means of resolving the problem regarding the role of diapycnal and isopycnal mixing in the deep ocean.

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1. Broecker, A. S. in *Symp. on Diffusion in Oceans and Fresh Waters* (ed. Ichiye, T.) 116–145 (Lamont-Doherty Geological Observatory, Palisades, 1965).
2. Moore, W. S. & Sackett, W. M. *J. geophys. Res.* **69**, 5401–5405 (1964).
3. Horibe, Y. (ed.) *Preliminary Report of the Hakuho Maru Cruise KH-80-2 and KH-82-1* (Ocean Research Institute, University of Tokyo, 1983).

4. Nozaki, Y. & Horibe, Y. *Earth planet. Sci. Lett.* **65**, 39–50 (1983).
5. Amin, B. S., Krishnaswami, S. & Somayajulu, B. L. K. *Earth planet. Sci. Lett.* **21**, 342–344 (1974).
6. Anderson, R. F. & Fleer, A. P. *Analyt. Chem.* **54**, 1142–1147 (1982).
7. Latimer, W. M. *Oxidation Potentials* 2nd edn (Prentice Hall, New Jersey, 1952).
8. Klinkhammer, G., Elderfield, H. & Hudson, A. *Nature* **305**, 185–188 (1983).
9. Cochran, J. K. thesis, Yale Univ. (1979).
10. Kaufman, A., Trier, R. M. & Broecker, W. S. *J. geophys. Res.* **78**, 8827–8848 (1973).
11. Li, Y. H., Feely, H. W. & Toggweiler, J. R. *Deep Sea Res.* **27**, 545–555 (1980).
12. Nozaki, Y., Tsubota, H. & Horibe, Y. *Earth planet. Sci. Lett.* **54**, 203–216 (1981).
13. Sarmiento, J. L., Feely, H. W., Moore, W. S., Bainbridge, A. E. & Broecker, W. S. *Earth planet. Sci. Lett.* **32**, 357–370 (1976).
14. Armi, L. *J. mar. Res.* **37**, 515–530 (1979).
15. Broecker, W. S. & Peng, T. H. *Tracers in the Sea* (Eldigio, Palisades, 1982).

Latitudinal dependency of geomagnetic polarity transition durations

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Palaeomagnetic records of the Matuyama–Brunhes polarity transition were obtained from seven, low sedimentation rate, deep-sea cores from the Pacific Ocean. The cores were taken near the 180° meridian and provide a latitudinal transect of transition records extending from 45.3° N to 33.4° S . Examination of these records suggests that low sedimentation rate cores may not be capable of recording the fine details of transitional field behaviour, but there are indications that these cores may, in fact, provide accurate records of the more general features of the reversal. Most notable of these features is that the duration of the transition is dependent on the site latitude, with durations at mid-latitudes being more than a factor of 2 longer than at equatorial latitudes.

Palaeomagnetic records of polarity transitions, particularly those of the Matuyama–Brunhes reversal, indicate that the field did not maintain a dipolar geometry during the reversal process^{1,2}. Higher-order symmetries, however, cannot be precisely defined because of the small number of detailed records and their poor geographical distribution.

There are inherent difficulties in obtaining an adequate data base because polarity reversals take a short time (geologically) to occur. Our approach to this problem has been to use micro-sampling techniques^{3–5} which allow us to obtain detailed records from deep-sea sediment cores which are characterized by low to moderate, yet uniform, sedimentation rates ($0.5\text{--}6 \text{ cm kyr}^{-1}$). This method greatly increases the possibility of obtaining a wide geographical distribution of records of the same reversal. The second advantage is that accumulation rates in cores are usually well determined and, therefore, it is possible to estimate rates and durations of processes recorded in the cores. A disadvantage is that there seem to be intrinsic problems in interpreting field behaviour from such low sedimentation rate cores⁴. These problems relate to the scale at which homogeneous magnetizations exist in the sediment whereby resolution of field behaviour does not seem to be proportionately improved by finer scale sampling. Therefore, we believe that the interpretation of such records should be limited to gross features of polarity transitions, such as the duration of the transition.

The cores were selected on the basis of earlier magnetostratigraphical studies (ref. 6 and N. Opdyke, personal communication) to investigate the possible latitudinal dependency of transition features. Seven cores from the Pacific Ocean, mostly distributed along the 180° meridian, were studied in an effort to minimize possible longitudinal effects on the transitional field geometry. One core from the Atlantic Ocean (V30-45), however, was also included (Table 1). In each of the cores the Matuyama–Brunhes reversal was sampled by sawing successive 4–5 mm slices from the dried, split half of the core. Each slice was subdivided to give three independent specimens per stratigraphical level for measurement using a two-axis cryogenic magnetometer. Pilot specimens from the cores were subjected

Table 1 Boundaries, transition zone thickness, sedimentation rate and transition duration for each core

Core	Lat.	Long.	Brunhes-Matuyama transition zone			Sedimentation rate* (cm kyr ⁻¹)	Standard error* (cm kyr ⁻¹)	Sedimentation rate† (cm kyr ⁻¹)	Transition duration* (kyr)
			Top (cm)	Bottom (cm)	Thickness (cm)				
RC10-182	45.37° N	177.5° E	820.7	832.3	11.6	1.10	0.063	1.13	10.5
V20-108	45.27° N	180.86° E	791.4	800.5	9.1	1.11	0.025	1.09	8.2
V20-107	43.24° N	181.48° E	525.3	532.3	7.0	0.73	0.044	0.74	9.6
V20-104	37.18° N	181.90° E	611.6	617.8	6.2	0.79	0.011	0.83	7.8
V30-45	6.18° N	340.44° E	622.3	624.8	2.3	0.53	0.020	0.722	4.3
RC15-21	1.33° N	227.41° E	830.6	835.7	5.1	0.98	0.015	1.11	5.2
RC9-119	23.23° S	188.42° E	661.5	664.1	2.6	0.69	0.073	0.87	3.8
RC9-114	33.41° S	194.98° E	875.4	882.7	7.3	1.2	—	1.2	6.1

* Calculation based on the depths of the Brunhes and Jaramillo reversals.

† Calculation with the origin included as a data point.

to progressive alternating field (a.f.) demagnetization studies at 2.5–5.0-mT increments up to 60 mT to identify the coercivity spectra of characteristic magnetization components. The remaining specimens were then demagnetized at the appropriate peak field level.

The results of blanket demagnetization at 10–20 mT produced records with inclinations in excellent agreement with the axial dipole field for the core site latitudes both above (normal) and below (reversed) the polarity transition zone. The internal consistency of the data and the agreement of the observed directions with axial dipole field values indicates that the sediment has been an accurate recorder of the geomagnetic field. The data are presented in Fig. 1 as virtual geomagnetic pole (VGP) latitude versus age (the ages were determined based on the sedimentation rates of the cores) to allow comparison of results from different site latitudes. As the cores were not oriented when taken, it was assumed that the mean declinations for intervals exhibiting normal (reversed) polarity inclinations were equal to 0° (180°). The entire declination record for each core was then

uniformly reoriented to reach the best agreement with this assumption. VGP positions were calculated from the unit-vector mean⁷ of the three observed directions at each stratigraphical level.

The criteria which are used to define the boundaries of polarity transition zones have an important effect on the calculated thickness especially when dealing with detailed records⁵. While defining the boundaries as the depths at which the VGP positions cross the $\pm 60^\circ$ or $\pm 45^\circ$ latitude^{2,8} provides an objective definition, it is clear in many of these records that transitional behaviour is recorded above and below these boundaries. The problem of defining the depths at which the transition is first (last) recorded becomes one of distinguishing a transitional direction from the reversed (normal) direction. We have, therefore, defined the transition zones as including the depth levels at which the VGPs exceed the circular standard deviation⁷ (c.s.d.) of the mean VGPs of the reversed and normal polarity zones. The boundaries defined in this manner and the resulting transition zone thickness for each core are listed in Table 1.

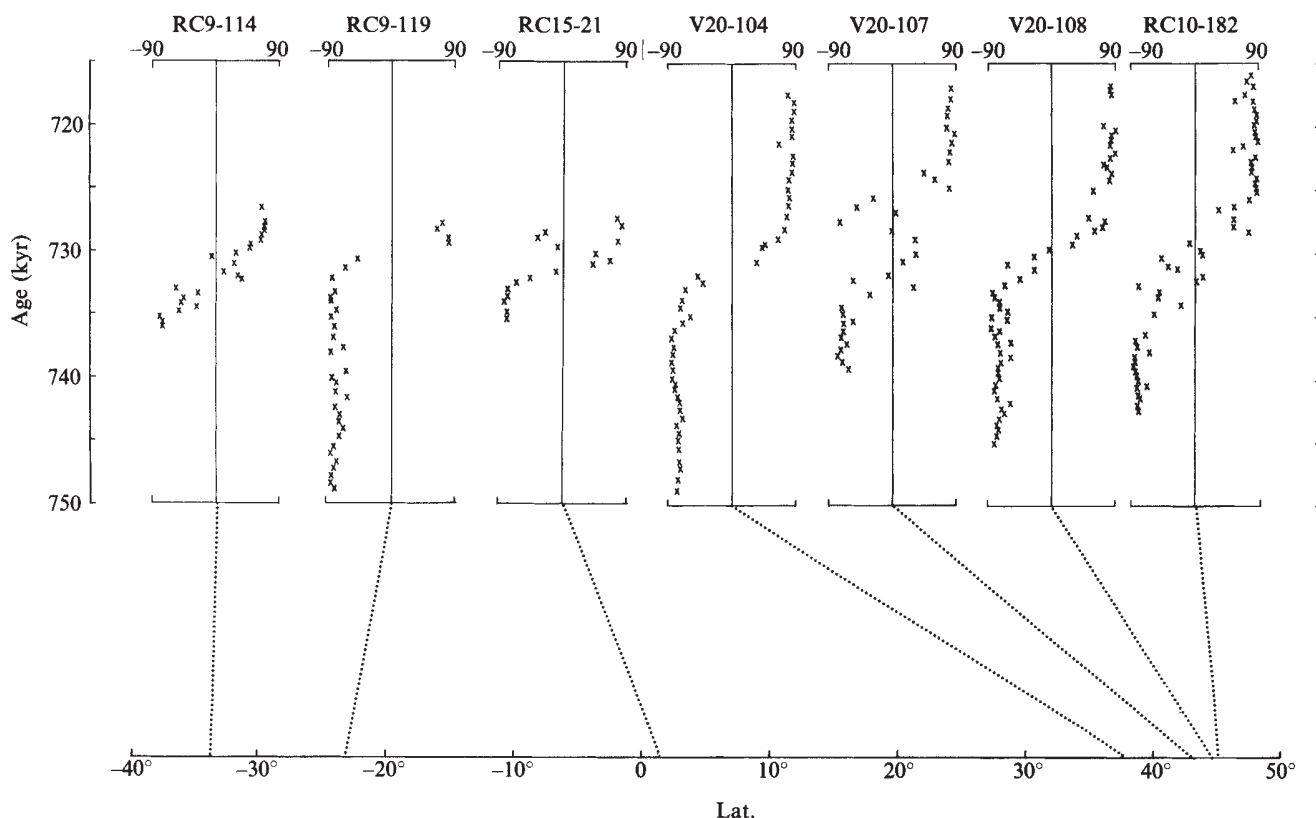


Fig. 1 VGP latitudes plotted against age for the seven cores. Each point represents the unit vector mean of the three VGPs determined at each stratigraphical level. The latitudes of the core site locations are indicated.

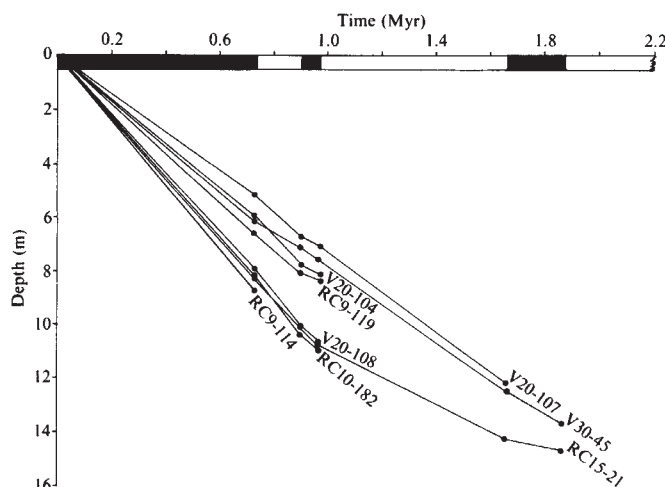


Fig. 2 Time-depth plot for the cores. The depths of the Brunhes, Jaramillo and Olduvai reversals are indicated.

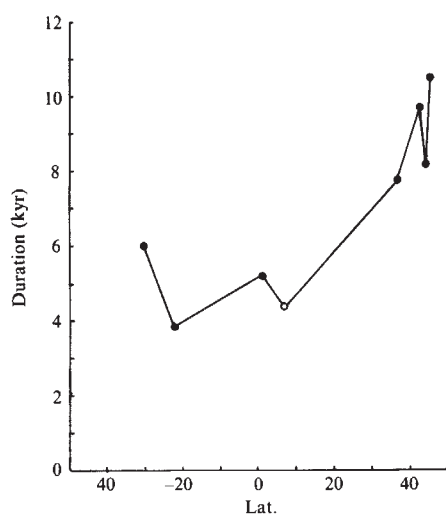


Fig. 3 Durations calculated for each core, plotted as solid circles versus core site latitude. The duration obtained from the Matuyama-Brunhes transition in V30-45 is plotted as an open circle.

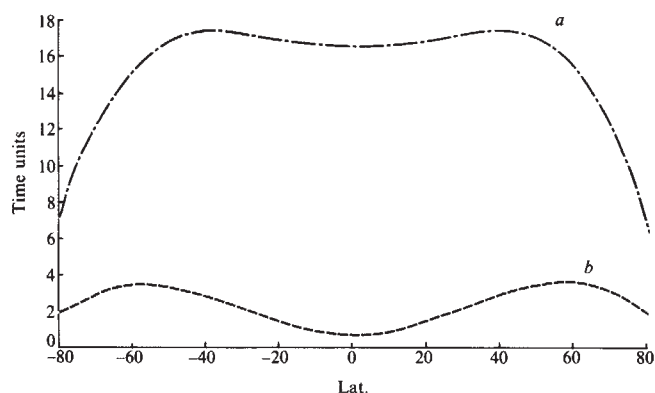


Fig. 4 Durations predicted by quadrupole (a) and octupole (b) versions of a zonal flooding model plotted in time units versus latitude.

Sedimentation rates for these cores were calculated based on the observed depths of polarity reversals (ref. 6 and N. Opdyke, personal communication). Only RC9-114 fails to penetrate a second reversal (Fig. 2). The tops of piston cores may not coincide with the sediment-water interface in some cases, and, therefore, the origin may not represent a valid data point. For three of the cores, RC10-182, V20-108 and V20-107, however, the rate calculated including the origin falls within the standard error of the rate calculated without the origin. This is a strong argument in favour of a constant rate of deposition for the past 0.98 Myr in these cores. The other three cores exhibit significant variation in the sedimentation rates when the origin is included. Therefore, we regard the best estimate of sedimentation rate across the Matuyama-Brunhes reversal (0.73 Myr) as that obtained by using the Brunhes and Jaramillo boundaries; durations of the polarity transitions were calculated accordingly (Table 1).

The largest source of error in the duration estimates probably lies in the sedimentation rate determinations. The standard errors of the sedimentation rate (Table 1) may be optimistic measures of the uncertainty in the sedimentation rates: for example, in the case of V20-104 the standard error suggests that the sedimentation rate of 0.79 cm kyr^{-1} is accurate to within $\pm 0.011 \text{ cm kyr}^{-1}$, but the actual variation in the sedimentation rate as determined between each of the three reversal boundaries is $0.088 \text{ cm kyr}^{-1}$ which can produce an uncertainty of 11% in the duration estimate. The uncertainty in sedimentation rates, combined with sampling resolution, however, is unlikely to account for the factor of 2 variation observed in the transition durations for these cores (Table 1).

The duration of the reversal appears to be a function of site latitude with durations from mid-northern latitudes about a factor of 2 longer than in the equatorial core (Fig. 3). The estimated duration of 4,300 yr from the Atlantic equatorial core (V30-45) is consistent with the estimates obtained from the Pacific cores, and suggests that the latitudinal dependency of durations may not be constrained to a particular longitudinal band. Southern Hemisphere coverage is confined to lower latitudes; an increase in duration with higher southern latitudes is suggested even though the shortest estimate was obtained from RC9-119 at 23° S . An apparent symmetry about the Equator (or at least about low latitudes) may exist, although it is clear that more records are needed to establish this trend fully. Those reversal duration estimates that are available are difficult to incorporate since, compared with the data presented here, most were obtained from very low-resolution records (sedimentation rates of only 0.16 cm kyr^{-1})⁹ or from records with poor control on sedimentation rates¹⁰. Even in cases where good time control is available^{11,12}, the criteria used to define the transition zone boundaries can make up to a factor of 2 difference in the estimated durations⁵. These difficulties, combined with the problems of differing sampling scales and laboratory techniques, provide the justification for considering the data presented here as an independent, homogeneous data set.

Some of the transition records are characterized by smooth progressions from reversed to normal polarity directions (for example, V20-104 and V20-108), but commonly more erratic behaviour is observed (V20-107 and RC15-21). The same erratic features are not recorded in nearby cores ($<200 \text{ km}$ away), casting doubt on their relation to geomagnetic field behaviour. These directions correspond to specimens in which the true transitional direction has not been properly isolated. Specimens from well outside the transition interval generally exhibit straightforward demagnetization behaviour, with characteristic directions clearly defined by linear trajectories that converge to the origin. Specimens from within these transition zones, however, are more often characterized by very complex magnetizations, making identification of a characteristic direction extremely difficult. In some specimens it was not possible to isolate confidently discrete components. Such behaviour is consistent with a model of varying lock-in depths for magnetic grains of slightly different sizes¹³. If the geomagnetic field during

a reversal was changing more rapidly than a specimen thickness (4–5 mm) of sediment was passing through the lock-in zone, then grains with overlapping coercivities within a thin stratigraphical level might have been locked-in at slightly different times thereby recording different field directions. A.f. demagnetization of such a specimen would not be able to isolate discrete components. Although this suggests that there may be problems with the detailed interpretation of transitional directions with regard to geomagnetic field behaviour in low sedimentation rate cores ($<1 \text{ cm kyr}^{-1}$), the lack of a correlation between sedimentation rate and duration suggests that the variation in transition duration is not strongly dependent on the magnetization process. Instead, the data presented here indicate that there is a dependence of transition duration on the site latitude as predicted by zonal transitional field models.

The sense of the latitudinal dependence of durations, however, is different from that predicted by Williams and Fuller's model of the Matuyama–Brunhes transitional field¹⁴. The model of combined low-order zonal terms seems to give a reasonable account for many of the observed inclination and intensity records across the reversal. It would, however, predict that durations from the Southern Hemisphere should be significantly longer than those from low latitudes and the Northern Hemisphere, whereas the data presented here show mid-northern latitude durations which are roughly twice as long as the equatorial and low-southern latitude durations.

It is not immediately obvious why this discrepancy exists between the Williams and Fuller model of the Brunhes–Matuyama reversal¹⁴ and the results presented here. Some insight, however, might be gained from examining two very simple representations of transitional field geometries, one dominated only by a zonal quadrupole field and another by a zonal octupole field. These transitional fields are modelled using Hoffman's⁸ technique which is based on the Parker–Levy^{15,16} approach to reversals. In this model the dipole field is simulated by placing a series of monopoles along a centred axis of length equal to the diameter of the Earth's core. Monopoles on opposite sides of the Equator are assigned opposite signs. The reversal is modelled by progressively reversing the signs of the monopoles, beginning at the Equator (octupolar transitional geometry) or at one end of the axis (quadrupolar) and evaluating the field observed at the Earth's surface for each of 200 configurations. For the 200 steps, each of which is arbitrarily defined as one time unit, the signs of two monopoles change. Relative durations are calculated by defining the boundaries of the transition as the points when the VGP's exceeded the normal and reversed polarity VGP's by 15° (by analogy to the c.s.d. of the core data).

Both the quadrupole (Fig. 4a) and octupole (Fig. 4b) versions of the model exhibit a symmetry about the Equator, with equatorial durations being shorter than mid-latitude durations. We do not know whether a similar symmetry exists in the data. However, the difference between the mid- and low-latitude durations shows a striking similarity with the durations from the deep-sea core records and is consistent with the dominance of the transitional field by low-order zonal terms. Unfortunately, the similar sense of variation from low to middle latitudes in both the octupole and quadrupole versions of this model makes it difficult to distinguish between the two with the given data set. A diagnostic feature, however, is that the quadrupole version yields durations at any given nonpolar latitude which are far greater than the octupole model. Thus in the quadrupole model this change is a small percentage of the maximum observed duration. When this difference is scaled using the core duration data, the quadrupole model predicts unrealistically long (70,000 yr) durations at mid-latitudes. On the other hand, because the difference is a much larger portion of the total in the octupole model, it predicts durations much closer to the observed values (6,000 yr) at mid-latitudes.

While there is a clear preference for the octupole model within the context of the narrow range of models considered here, transitional fields are, of course, likely to be more complex than

that described by a single zonal term. Until the pattern of duration with site latitude is more fully established, it will be difficult to use the duration data to assess the contribution of addition terms. In particular, duration as well as transitional direction and intensity data from the Southern Hemisphere will be critical in constraining transitional field models.

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- Hillhouse, J. & Cox, A. *Earth planet. Sci. Lett.* **29**, 51–64 (1976).
- Fuller, M., Williams, I. & Hoffman, K. *Rev. Geophys. Space Phys.* **17**, 179–203 (1979).
- Kawai, N., Otofui, Y. & Kobayashi, K. *J. Geomagn. Geoelectr.* **28**, 395–412 (1976).
- Clement, B., Kent, D. & Opdyke, N. *Phil. Trans. R. Soc. A306*, 113–119 (1982).
- Clement, B. & Kent, D. *J. geophys. Res.* **89**, 1049–1058 (1984).
- Ninkovitch, D., Opdyke, N., Heezen, B. & Foster, J. *Earth planet. Sci. Lett.* **1**, 476–492 (1966).
- Fisher, F. *Proc. R. Soc. A217*, 295–305 (1953).
- Hoffman, K. *Science* **196**, 1329–1332 (1977).
- Harrison, C. & Somayajulu, B. *Nature* **212**, 1193–1195 (1966).
- Niitsuma, N. *Tohoku Univ. Sci. Rep. 2nd Ser.* **43**, 1–39 (1971).
- Valet, J.-P. & Laj, C. *Earth planet. Sci. Lett.* **54**, 53–63 (1981).
- Hammond, S., Seyb, S. & Theyer, F. *Earth planet. Sci. Lett.* **44**, 165–175 (1979).
- Tucker, P. *Geophys. J. R. astr. Soc.* **63**, 149–163 (1980).
- Williams, I. & Fuller, M. *J. geophys. Res.* **87**, 11,657–11,665 (1982).
- Parker, E. *Astrophys. J.* **158**, 815 (1969).
- Levy, E. *Astrophys. J.* **171**, 621 (1972).

Thermoluminescence dating of periods of loess deposition and soil formation in Normandy

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The least incomplete records of Pleistocene climate change come from the study of deep-sea sediments in which the stratigraphic record of oxygen isotope variations, reflecting terrestrial ice volume, is the primary correlation medium. On continents, the available record is far less complete because many of the characteristics of glaciation are erosional rather than depositional. The areas of loess deposition may provide the best deposits in which to develop relatively complete regional stratigraphies that may be compared with the deep-sea record^{1,2}. In this study we use thermoluminescence (TL) dating of loess, and of soil developed on loess, to test this model. We have confined ourselves to the last complete glacial cycle within which the reproducibility of the age determinations is similar to the time resolution of most deep-sea core records.

The selected site is in a disused brick pit (now used as the town's rubbish tip) at Saint Romain on the plateau of the Pays de Caux, about 20 km east of Le Havre. It is typical of the Normandy loess sites and a reference section for the 'limons à doublets'. The section totals 15 m but we are concerned with the upper 5 m. Figure 1 shows this part of the section, which was sampled for TL dating (by A.G.W. and N.J.S.) in May 1982 during and after a field excursion (led by J.-P.L.).

Sixteen samples (TL reference numbers QTL20a–p) were taken from the section which has been described elsewhere^{3,4}. The lowest samples p (450 cm) and o (420 cm) are from the 'older loess' which lies below the Saint-Romain soil. The latter is distinguished from the underlying loess by its redness, its higher clay content and its polyhedral structure. It was first thought to be interstadial³ but subsequently has been described as a leached, brown interglacial soil⁴. It is overlain by a brown, lamellar, early Weichselian loam⁴ containing evidence of slope wash and solifluxion processes. Three samples k (300 cm), j

Table 1 Thermoluminescence results and radioactivity data for Saint-Romain

Depth	No.	Bulk α -count rate ($\text{ks}^{-1}\text{cm}^{-2}$)	Th (p.p.m.)	U (p.p.m.)	Sealed Unsealed	K_2O (%)	Dose rate (mGy yr^{-1})	$\overline{\text{ED}} \pm \sigma$ (Gy)	Age (kyr)	% CaCO_3
30	a	0.744 ± 0.018	6.4 ± 1.4	4.4 ± 0.4	1.02	1.96 ± 0.01	3.91	49.3 ± 3.5	12.6 ± 1.3	3.9
60	b	0.805 ± 0.017	10.1 ± 3.8	3.8 ± 0.5	0.96	1.88 ± 0.005	4.04	58.5 ± 2.1	14.4 ± 1.3	7.3
90	c	0.947 ± 0.015	10.6 ± 1.4	4.8 ± 0.4	1.03	1.81 ± 0.009	4.45	61.2 ± 1.6	13.7 ± 1.2	1.0
120	d	0.931 ± 0.013	9.9 ± 1.3	4.9 ± 0.4	1.09	1.81 ± 0.005	4.39	62.4 ± 2.0	14.2 ± 1.3	1.5
150	e	0.759 ± 0.013	9.5 ± 1.2	3.6 ± 0.4	1.02	1.80 ± 0.005	3.84	42.7 ± 1.6	11.1 ± 1.0	2.6
178	f	0.862 ± 0.012	11.3 ± 1.1	3.9 ± 0.4	1.01	1.77 ± 0.02	4.15	68.1 ± 2.6	16.4 ± 1.5	2.6
182	g	0.693 ± 0.011	11.3 ± 1.2	2.5 ± 0.4	1.03	1.47 ± 0.01	3.39	254 ± 1.0	75 ± 6.5	2.3
210	h	0.743 ± 0.014	9.6 ± 1.4	3.4 ± 0.4	0.97	1.50 ± 0.01	3.57	288 ± 5.0	80 ± 7	3.3
240	i	0.717 ± 0.016	9.0 ± 1.5	3.4 ± 0.5	0.87	1.46 ± 0.005	3.46	307 ± 2.0	88 ± 8	3.7
270	j	0.828 ± 0.018	9.6 ± 1.7	4.1 ± 0.5	0.95	1.45 ± 0.005	3.81	329 ± 1.9	86 ± 8	3.0
300	k	0.813 ± 0.018	9.8 ± 1.7	3.9 ± 0.5	0.96	1.61 ± 0.01	3.88	323 ± 4.0	83 ± 7	4.2
330	l	0.736 ± 0.016	8.9 ± 1.6	3.5 ± 0.5	0.97	1.69 ± 0.01	3.69	442 ± 2.0	120 ± 10	7.6
360	m	0.764 ± 0.017	7.2 ± 1.5	4.3 ± 0.5	0.91	1.71 ± 0.007	3.79	420 ± 4.0	111 ± 10	3.9
390	n	0.702 ± 0.015	7.7 ± 1.4	3.6 ± 0.4	0.98	1.65 ± 0.01	3.55	407 ± 11	114 ± 10	3.0
420	o	0.726 ± 0.012	7.4 ± 1.0	3.9 ± 0.3	1.10	1.68 ± 0.007	3.65	457 ± 19	125 ± 11	3.7
450	p	0.626 ± 0.012	7.8 ± 1.2	3.0 ± 0.4	1.03	1.67 ± 0.01	3.32	462 ± 16	139 ± 12	2.0

(270 cm) and i (240 cm) were taken from this sediment, the upper part of which is a marbled soil with orange and grey flecks. Above this is a weakly bedded loess from which two samples, h (210 cm) and g (182 cm), were taken. At the top of the loess is a soliflucted, tongued horizon with fossil ice wedges which was very clearly marked in the section we sampled. Above this is finely laminated loess, known as *limons à doublets*. We collected one sample only 2 cm above the hiatus, f (178 cm), and then another five samples at 30 cm intervals up to the base of the modern soil, e (150 cm), d (120 cm), c (90 cm) and a (30 cm). All depths are measured from the top of the modern soil. Heavy mineral analyses have been carried out on all the deposits⁴. An earlier study of the palaeomagnetic properties of four samples from beneath the upper palaeosol⁵ indicated normal polarity, but no measurements were made higher up the section. No pollen was found in the section, but detailed studies have been made of the palaeosol micromorphology.

Three methods, described in detail elsewhere⁶, for obtaining the equivalent dose (ED) used in TL dating were used for the uppermost six samples (QTL20a–20f). The only difference in the laboratory procedure is the use of a Schott UG11 filter, instead of a Corning 5-58 filter, as suggested by Debenham and Walton⁷. This enhances the TL signal observed from the feldspar component relative to that of quartz. Note that, whilst feldspars of volcanic origin show anomalous fading⁸, this phenomenon is rarely found in sedimentary feldspars. Another technical innovation is the use of 1 min preheating at 230 °C to produce the glow curves shown in Fig. 2.

The three methods agreed well; for example, for QTL20f the values were 67.6 ± 3 , 71 ± 7 and 68.6 ± 11 Gy. The weighted mean EDs are given in Table 1 and used in the age calculation. Below 180 cm the samples can only be dated by one of the methods: $N + SL + \beta$ in which the TL signal is regenerated after a long sunlamp exposure in the laboratory. The other methods cannot

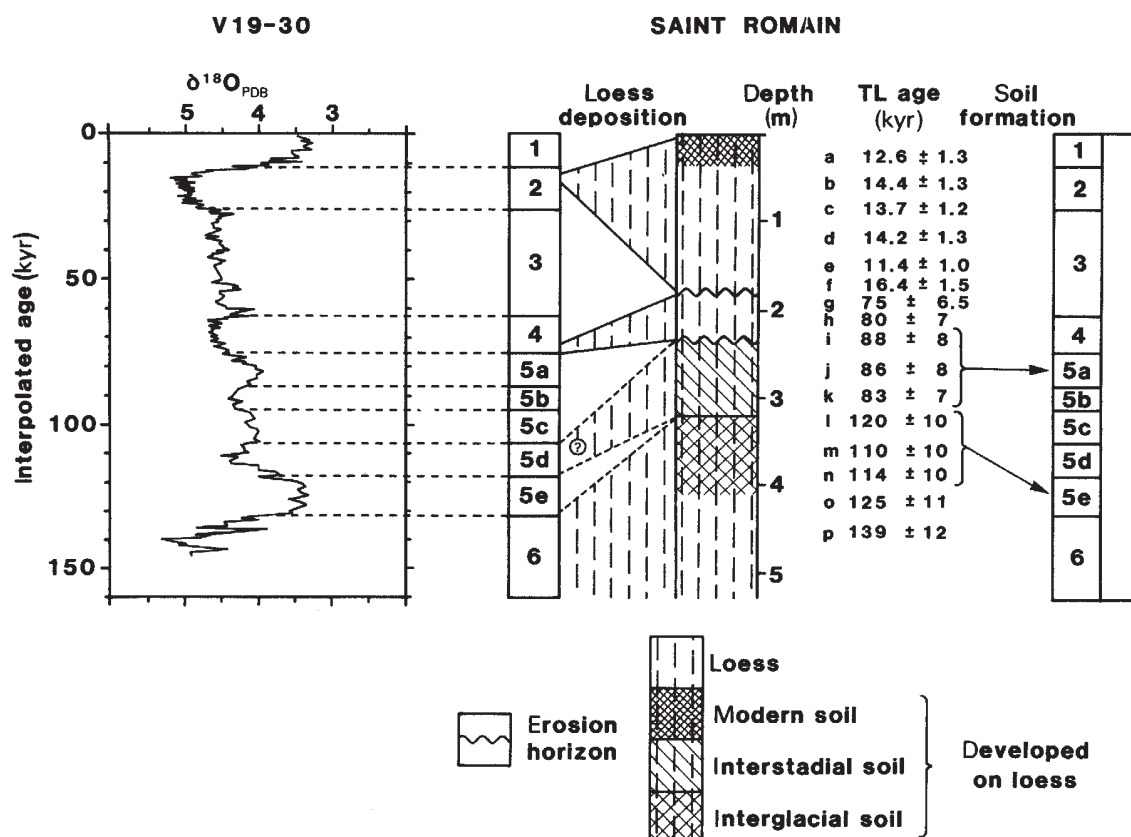


Fig. 1 Correlation of loess deposition and soil formation, as dated by TL, and the marine oxygen isotope record.

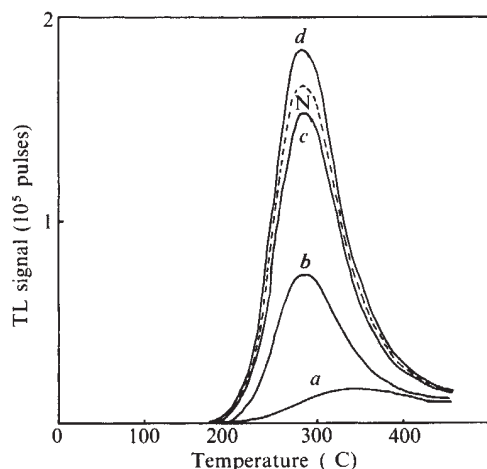


Fig. 2 TL glow curves obtained using a 1-min preheat at 230 °C for sample QTL20h. N is the natural TL, a is the natural TL after 450 min exposure to a sunlamp and b, c and d are the same as a but have received doses of 130, 260 and 325 Gy respectively.

be applied since the TL response becomes non-linear for doses above 200 Gy, and they both rely on a linear dose response (for example, in QTL20g the TL sensitivity at the level of the natural TL is only 60% of its initial sensitivity).

The contributions of the uranium and thorium decay chains to the natural environment were obtained by thick-source α counting⁹ each sample. The counts were performed both unsealed and sealed to look for radon emanation; only three samples show significant differences, two increased and one decreased. The ^{40}K contribution was obtained from the total K_2O content as determined by atomic absorption analysis. A water content (defined as wt water/wt dry sediment) of 0.20 ± 0.05 was used to correct the annual dose rate and allow for the fraction of the dose which is absorbed in the water rather than in the minerals. This is the range found for the water content of undisturbed loess in northern France at a depth >1 m from the surface¹⁰. Table 1 gives analytical data and age estimates. The errors are calculated using the rigorous error analysis developed for TL dating of pottery⁹. The TL ages may be compared with the oxygen isotope stages¹¹; the last interglacial being represented by substage 5e, the extremes of the last glacial by stages 4 and 2, which are separated by the less cold stage 3, and the Holocene by stage 1. The age 115 ± 10 kyr estimated for the Saint Romain soil, from three statistically indistinguishable values, is in excellent agreement with the accepted age of substage 5e (about 126 kyr to 118 kyr in Fig. 1) as well as with the Gascoyne *et al.*¹² uranium series date for the Ipswichian *Hippopotamus* fauna in Yorkshire, UK (120 ± 6 kyr). The underlying loess yields estimates consistent with accumulation in late stage 6, the preceding glacial stage.

The three dates for the brown loam are again indistinguishable and show that it formed during substage 5a on a loess that must have accumulated during substage 5d and/or 5b. Wintle and Catt¹³ have shown that the A horizons of soils developed on loess can be dated by TL. It is thought that zeroing of the TL signal that had accumulated in the loess is caused by exposure to sunlight following the mineral grains being brought to the surface by bioturbation. Therefore, we are unable to estimate the duration of the soil-forming episode. The use of the phrase

'early Weichselian' for such an interstadial soil in this part of France is consistent with the correlation of the last interglacial with substage 5e and with the beginning of the Weichselian ~ 110 kyr. This interpretation provides for interstadials which were of sufficient intensity to produce soils but which are substantially beyond the present upper limit of ^{14}C dating¹⁴. The data presented here supports Woillard and Mook's¹⁵ correlation of the Saint Germain I and Saint Germain II warm stages in the Grande Pile sequence with substages 5a and 5c.

Above this loam the loess yielded age estimates of 75 ± 6.5 kyr and 80 ± 7 kyr, suggesting correlation with stage 4, a short but intense glacial stage in the marine oxygen isotope record. The 75-kyr date was obtained 2 cm below an obvious hiatus in the section. The sample collected in the loess 2 cm above the hiatus has a date of 16.4 ± 1.5 kyr giving a gap of about 60 kyr. The top 1.8 m of loess was deposited rapidly, at a rate of at least 60 cm kyr^{-1} .

Our dates suggest that it accumulated late in stage 2 (Fig. 1) either at maximum ice advance or early in the succeeding deglaciation. The dating of the beginning of deglaciation in the marine record is controversial, with opinions ranging from 16 to 13 kyr (ref. 16) so that the loess, for which our dates yield a mean of 13.8 kyr, may, in fact, have accumulated after the sea level had risen by a significant extent. However, it was certainly earlier than the stage 2-1 boundary whose age is well established close to 10.6 kyr (ref. 17).

The data suggest that loess accumulated rapidly so that, at least in this section, it is very unlikely that as much as 10% of late Pleistocene time was occupied by intervals of loess accumulation. This suggests that loess accumulation may, in fact, have occupied only as small a proportion of Pleistocene time as interglacials did². On the other hand, there may be other sections in the region which fill some of the apparent time gaps in the Saint Romain section. It is puzzling that we find no loess dating to any part of the interval between 80 and 20 kyr, as we have dated a significant accumulation of loess in Belgium with an age around 57 kyr. Thus, data from more sections may well modify the present picture.

The preservation of evidence for interstadial and interglacial soil-forming intervals greatly enhances the value of a loess section, but other breaks may represent short or long intervals of intermediate climate. Correlation will be more accurately achieved by TL dating loess bodies in different sections than by regarding the breaks between them as time-horizons for which correlation from site to site is attempted.

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- Kukla, G. J. *Earth-Sci. Rev.* **13**, 307-374 (1977).
- Emiliani, C. *Ann. N.Y. Acad. Sci.* **95**, 521-536 (1961).
- Lautridou, J. P. *Bull. Cent. Geomorphol. Caen* **2**, 1-55 (1968).
- Lautridou, J. P. *Bull. Cent. Geomorphol. Caen* **27**, 1-88 (1982).
- Biquand, D. & Lautridou, J. P. *Bull. Ass. Fr. Etude Quat.* **16**, 75-81 (1979).
- Wintle, A. G. & Prószyńska, H. *PACT* **9**, 547-554 (1983).
- Debenham, N. C. & Walton, A. J. *PACT* **9**, 531-538 (1983).
- Wintle, A. G. *Nature* **245**, 143-144 (1973).
- Aitken, M. J. *Physics and Archaeology* (Clarendon, Oxford, 1974).
- Audric, T. & Bouquier, L. *Q. Jl Engng Geol.* **9**, 267-277 (1976).
- Shackleton, N. J. *Proc. R. Soc. B174*, 135-154 (1969).
- Gascoyne, M., Currant, A. P. & Lord, T. C. *Nature* **294**, 652-654 (1981).
- Wintle, A. G. & Catt, J. A. *J. Soil Sci.* (submitted).
- Groote, P. M. *Science* **200**, 11-15 (1978).
- Woillard, G. M. & Mook, W. G. *Science* **215**, 159-161 (1982).
- Ruddiman, W. F. *Quat. Res.* (in the press).
- Mangerud, J., Lie, S. E., Furnes, H., Kristiansen, I. L. & Lomo, L. *Quat. Res.* **21**, 85-104 (1984).

Calcium phosphate granules in muscle cells of *Nephtys* (Annelida, Polychaeta)—a novel skeleton?

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Analyses of various marine polychaete worms (phylum Annelida) reveal that members of the family Nephtyidae are exceptional in containing very high concentrations of calcium and phosphorus. We report here that in the genus *Nephtys*, the two elements form rounded granules packing the sarcoplasmic core of obliquely striated muscle cells. Intracellular calcium granules rich in phosphorus are found throughout the animal kingdom and occur in a wide range of tissues^{1,2}. Although some examples of granules in muscle tissue have been recorded (as in *Syllis proventriculus*³), as far as we are aware, none appears in the abundance found in *Nephtys*.

Nephtyid polychaete worms are found worldwide and are one of the commonest carnivores inhabiting sediments from the intertidal zone to abyssal depths. The family Nephtyidae comprises about 100 species; these are grouped in four genera but most belong to the genera *Nephtys* and *Aglaophamus*⁴. To date, 11 species have been analysed and all show a similarly high content of calcium and phosphorus: on a dry weight basis, whole bodies contain 2–5% calcium and 2–4% phosphorus (Table 1). In contrast, non-nephtyid polychaetes generally contain less than 1% of both elements (38 species in 25 families have been examined). The observations presented below relate to two European species, *Nephtys hombergi* and *Nephtys caeca*, but it is thought that all nephtyids have the same basic structure. *N. caeca* is the largest of the European species, growing to 25 cm long, and has a lifespan of up to 7 yr; *N. hombergi* is smaller, living up to 5 yr⁵.

The reason for the high calcium and phosphorus contents of *Nephtys* is readily apparent when any muscle tissue is examined. In fractured body sections, the main longitudinal blocks of muscle that form the dorsal and ventral body wall, for example (Fig. 1a,b), contain numerous granules disposed in elongate bundles (Fig. 1c). Teased fibre preparations show that these granules form ribbon-like structures (Fig. 1d) extending throughout the middle four-fifths of most fibres which measure up to 1 mm long. The closely packed arrangement of the granules gives the ribbons a 'cobble-stone' appearance. The granules are somewhat variable in shape and size but most are oval to elongate, 0.5–1.0 µm in diameter and up to 3 µm long, for the most part aligned along the length of the fibre.

Thin sections of *Nephtys* muscles show that the fibres are flattened and obliquely striated, similar to other polychaete fibres (*Nereis*⁶, *Glycera*⁷ and *Sabella*⁸), but differing in having a sarcoplasmic core filled with granules and mitochondria (Fig. 2). The hardness of the granules causes them to fracture on sectioning (Fig. 2a); no internal structure, such as concentric rings, is apparent. When decalcified, most granules can be seen to be membrane-bound with a diffuse internal matrix, often with a distinct, near-central 'nucleus' (Fig. 2c,d). The close juxtaposition of the granules and mitochondria is a conspicuous feature.

Opaque inclusions in the muscle fibres of *Nephtys* were recorded by several early investigators, notably Claparède⁹ who thought they might be composed of fat. However, granules isolated from tissue homogenates prove to be composed largely of calcium and phosphorus (Fig. 1e; Table 2). The mean Ca/P atom ratio of *N. caeca* granules exceeds 1.6 (Table 2), suggesting that the principal component of the granules is hydroxyapatite [$\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ (Ca/P = 1.67), possibly with an admixture of tricalcium orthophosphate, $\text{Ca}_3(\text{PO}_4)_2$ (Ca/P = 1.5). In H_2O_2 -treated granules, calcium and phosphate together account for about 90% of the residue after ashing; other components include

Table 1 Calcium and phosphorus concentrations in species of *Nephtys*, *Nereis* and *Glycera*

Species	Site	Tissue	Concentration (% dry tissue)	
			Ca	P
<i>Nephtys hombergi</i>	A	Whole body	5.14	3.32
<i>Nephtys cirrosa</i>	A	Whole body	4.75	3.44
<i>Nephtys caeca</i>	B	Whole body	4.13	2.19
<i>Nephtys caeca ciliata</i>	C	Whole body	3.16	2.13
	C	Dorsal body wall*	10.15	6.54
	C	Ventral body wall*	10.65	6.88
<i>Nereis diversicolor</i>	D	Whole body	0.154	0.567
<i>Nereis virens</i>	E	Whole body	0.136	0.655
<i>Glycera gigantea</i>	F	Whole body	0.608	0.830
<i>Glycera convoluta</i>	G	Whole body	0.429	0.677

Sites: A, Place Cove, Fal estuary; B, Salcombe; C, Yealm estuary; D, Avon estuary (Devon); E, Plymouth; F, Eddystone shell gravel; G, Par Sands. Worms were maintained in acid-washed sand in seawater for several days, then transferred to seawater for 1 day. Worms and tissues were dried at 80 °C, wet-ashed in HNO_3 , and, after evaporation, dissolved in 50% HCl subsequently diluted to give 1M HCl. Metal analyses were performed by flame atomic absorption; an ammonium molybdate method¹⁴ for orthophosphate was used for phosphorus determinations.

* 10 Mid-body segments.

magnesium and strontium (Table 2) and a significant amount of chlorine (Fig. 1e).

The presence in *Nephtys* of these phosphorus-rich calcium granules poses interesting problems as to their formation and function. Their formation seems to involve mitochondria as calcium phosphate deposits have been detected in mitochondria in the muscle fibres of both adult and juvenile worms (Fig. 2b) and, further, the mitochondria are usually in close contact with the granules (Fig. 2c,d).

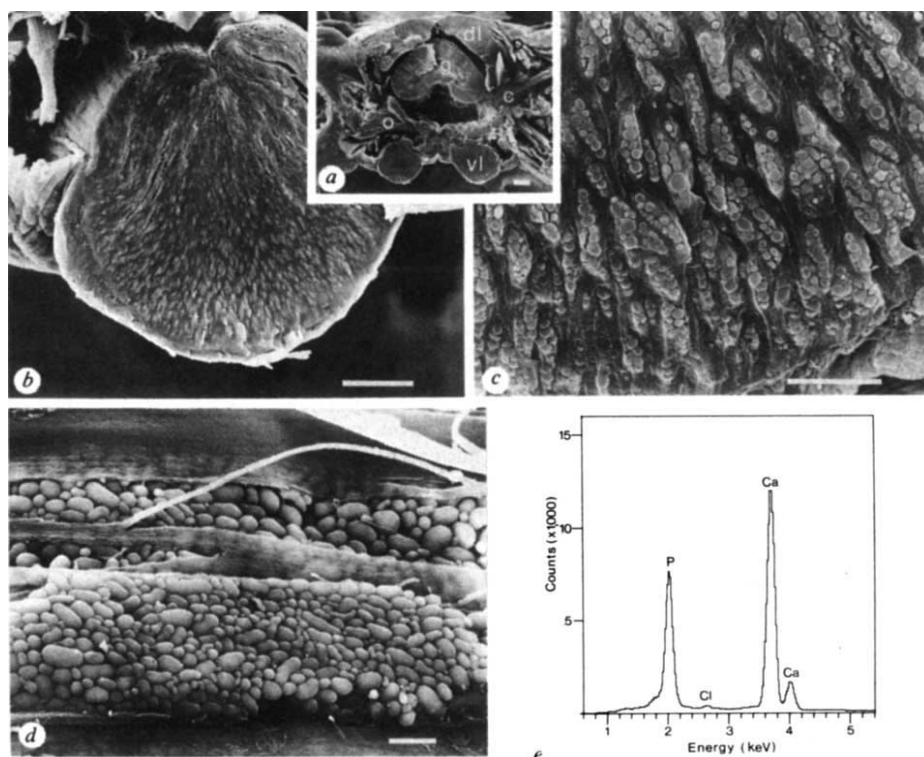
The granules account for about 10% of the wet muscle mass or ~50% of the dried tissue (assuming muscle forms 70% of the body wall and contains most of its calcium—see Table 1); in such quantity, it is doubtful that the granules are simply a means of storing calcium phosphates. Further, the granules do not seem to be involved primarily in the detoxication of trace metals as worms from a highly metallic site (Restronguet Creek, Fal estuary¹⁰) do not contain enhanced calcium and phosphorus concentrations and, also, there is no evidence for accumulation with age because a similar range of levels is found in all size

Table 2 Composition of *Nephtys* muscle granules

	Concentration (% of ash)	
	Soluene-treated	H_2O_2 -treated
Ca	34.46	36.38
P	16.42	17.49
PO_4	50.34	53.64
Mg	1.75	0.275
Sr	0.0569	0.0514
Ca/P atom ratio	1.62	1.61

Large specimens, ~15 cm long, of *N. caeca* were used as a source of granules for this analysis. Whole specimens, previously deep-frozen, were placed in near-boiling distilled water for 15 min to facilitate removal of muscle. After homogenization and centrifugation, adhering organic material was removed from the granules by treatment with Soluene-100 (ref. 1) or H_2O_2 (30% w/v) at 50 °C for 65 h. Soluene-treated granules were washed in toluene, followed by ethanol; H_2O_2 -treated granules were washed in water. Both sets were then dried at 85 °C and converted to ash overnight at 450 °C. The ash was dissolved in 1M HCl before analysis (see Table 1). Conversion to ash resulted in a weight loss of 8.35% in Soluene-treated granules and of 20.57% in H_2O_2 -treated granules; ashing did not appear to change the Ca/P atom ratio significantly.

Fig. 1 *a-d*, Scanning electron micrographs of *N. hombergi* tissues. Freeze-fractured transverse sections of: *a*, mid-body segment, anterior view, showing main muscles (*c*, chaetal sac; *dl*, *vl*, dorsal and ventral longitudinal muscles, respectively; *g*, gut; *o*, oblique muscle; *p*, parapodial muscle); *b, c*, ventral longitudinal muscle showing arrangement and orientation of granule ribbons. *d*, Side view of two teased fibres showing the shape and size of granules in ribbons. Scale bars: *a*, 100 μ m; *b*, 50 μ m; *c*, 10 μ m; *d*, 2 μ m. All tissues were taken from 30–40 mm-long specimens fixed in glutaraldehyde and formalin in seawater, post-fixed in osmium, acetone-dehydrated and critical point-dried. *a-c*, Freeze-fractured after post-fixation; *d*, teased after drying; *e*, typical spectrum obtained by X-ray microanalysis of *N. caeca* granules (H_2O_2 -treated). K-line peaks for Ca, P and Cl are indicated.



groups. Rather, the quantity and arrangement of the granules suggest that their role is structural.

The granules are present in *Nephtys* of all sizes, even in recently settled juveniles measuring 1 mm long, thus they seem to be an integral part of the musculature with an important role in movement. Swimming and burrowing in relation to the muscu-

lature of *Nephtys* have been extensively investigated^{11,12}. Members of the genus *Nephtys* are among the most active of burrowing polychaetes: *Nephtys cirrosa*, for example, has been shown to be capable of burrowing 30 times faster than the ragworm *Nereis diversicolor*¹³, this difference is perhaps not surprising as *Nephtys* is more muscular and less compressible than *Nereis*¹². The robust character of *Nephtys* may derive, at least in part, from the granular packing of all muscles. The granules form numerous ribbons that interleave with the contractile elements. On contraction the ribbons will be compressed, thereby imparting a degree of rigidity to the whole muscle. Most ribbons in the main dorsal and ventral longitudinal muscles are flattened in the vertical plane (Fig. 1*b*), an arrangement that permits the lateral sinusoidal movements used in locomotion but resists dorso-ventral flexion, no doubt an advantage when effecting penetration of sediments.

No comparable accumulation of calcium phosphate in muscle cells is known in the animal kingdom. We suggest that a novel flexible skeleton has evolved in *Nephtys* as an integral part of its highly successful burrowing mechanism.

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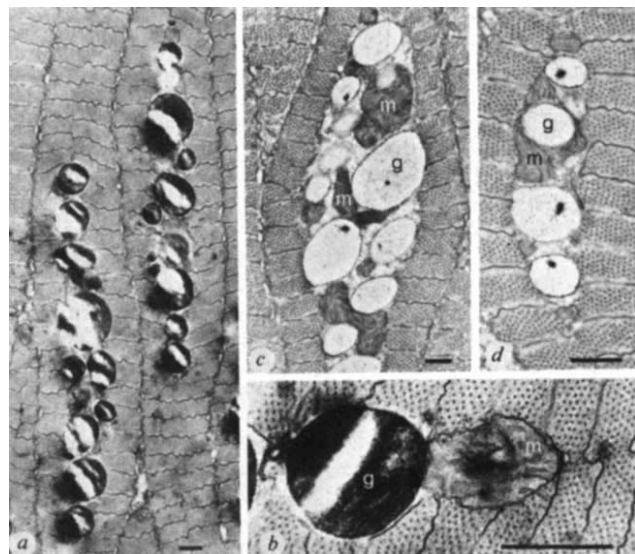


Fig. 2 Transmission electron micrographs of transverse sections of dorsal longitudinal muscle from *N. hombergi*. *a*, Central portions of two fibres showing myofibrils surrounding granule ribbons within the sarcoplasmic core. *b*, Granule (*g*) and mitochondrion (*m*) with electron-dense calcium phosphate deposit (determined by X-ray microanalysis). *c, d*, Appearance of granules and associated mitochondria in section after decalcification. All scale bars, 0.5 μ m. Muscle was fixed in glutaraldehyde and formalin in seawater, post-fixed in osmium, acetone-dehydrated and stained with lead citrate after sectioning in Spurr resin; sections *c, d* were decalcified with 2% ascorbic acid after post-fixation¹⁵.

1. Simkiss, K. *Symp. Soc. exp. Biol.* **30**, 423–444 (1976).
2. Mason, A. Z. & Nott, J. A. *Aquat. Tox.* **1**, 239–256 (1981).
3. Ponsolle, L., Wissocq, J.-C. & Galle, P. *Cytobiologie* **9**, 169–179 (1974).
4. Fauchald, K. *Nat. Hist. Mus. Los Angeles County, Sci. Ser.* **28**, 1–190 (1977).
5. Olive, P. J. W. *J. mar. biol. Ass. U.K.* **57**, 133–150 (1977).
6. Alohan, F. I. & Huddart, H. *Tissue Cell* **13**, 525–534 (1981).
7. Rosenbluth, J. J. *Cell Biol.* **36**, 245–259 (1968).
8. Kryvi, H. *Norw. J. Zool.* **22**, 67–69 (1974).
9. Claparède, E. *Mém. Soc. Phys. Hist. nat. Genève* **19–20**, 1–500 (1868).
10. Bryan, G. W. & Gibbs, P. E. *Occ. Publ. mar. biol. Ass. U.K.* **2**, 1–112 (1983).
11. Clark, R. B. & Clark, M. E. *Q. Jl. microsc. Sci.* **101**, 149–176 (1960).
12. Mettam, C. J. *Zool.* **153**, 245–275 (1967).
13. Trevor, J. H. *Estuar. cost. mar. Sci.* **6**, 605–619 (1978).
14. *Phosphorus in Waters, Effluents and Sewages*, 1980, 30 (HMSO, London, 1981).
15. Dietrich, H. F. & Fontaine, A. R. *Stain Technol.* **50**, 351–353 (1975).

Posterior-to-anterior transformation in *engrailed* wing imaginal disks of *Drosophila*

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Segments in the *Drosophila* adult are divided into clonally distinct anterior and posterior compartments¹⁻⁵. Mutations at the *engrailed* locus can affect the pattern of cuticular structures in the posterior compartments of segments, but have no obvious effect on anterior structures⁴⁻¹²; for example, bristles that are normally seen only on the anterior wing margin in wild-type flies can be found on the posterior margin of *engrailed* wings. These and clonal analysis data led to the hypothesis that *engrailed* causes a transformation of posterior to anterior identity in the wing cells⁶. Despite some striking examples of this transformation, a common *engrailed* phenotype is the disruption or elimination of posterior pattern elements, without a clear replacement by anterior structures⁸⁻¹³; this, together with indications that localized cell death can mimic some of the observed posterior-to-anterior transformations¹⁴, has led some investigators to question the original *engrailed* hypothesis¹². Recently, monoclonal antibodies displaying region-specific binding patterns on the wing imaginal disk have been described¹⁵⁻¹⁷, and one of these antibodies in particular provides a novel probe for the *engrailed* phenotype in the larval precursors of the adult wing. Here I compare the antibody binding patterns on *engrailed* and wild-type wing disks. The results strongly support the notion that *engrailed* mutations cause a posterior-to-anterior transformation in these cells.

In late third-instar larvae, the wing imaginal disk is a flattened epithelial sac (Fig. 1). Most of the cells that will contribute to recognizable adult structures can be found on the columnar epithelium on one side of the disk, and the pattern of the adult structures can be fate-mapped onto this face of the disk in two dimensions¹⁸. From this map, the axes of the disk (that is, anterior/posterior, dorsal/ventral, proximal/distal) are defined with respect to the locations of the structures in the adult fly. A large central pouch contains the precursors of the adult wing blade, with the more peripheral regions of the disk giving rise to proximal thoracic structures. The individual cells are morphologically very similar in different regions of the disk¹⁹. Recently, however, three classes of position-specific (PS) monoclonal antibodies have been isolated which show non-uniform patterns of binding to the wing disk epithelium^{16,17}. These antibodies recognize biochemically related complexes of cell-surface glycoproteins^{16,20} and the expression of these antigen complexes appears to be related not to the type of adult structure that a particular disk region is specified to make, but rather to position in the epithelium. Antibodies of the PS2 category show distinguishable patterns of binding to the anterior and posterior halves of the wing imaginal disk. These patterns are reproducible in detail and, unlike other disk cell markers²¹, do not seem to be altered by genetic changes that do not affect the adult pattern. For example, the mature wing disk immunofluorescence pattern is similar in related species of *Drosophila* and in mutants, such as *Minutes*, that alter growth without altering the adult pattern (unpublished observations). Thus, PS2 antibodies provide a useful marker for the effects of *engrailed* mutations in the undifferentiated epithelium.

In the wild-type wing pouch, there is strong PS2 immunofluorescence on the ventral part of the disk, with some weakly

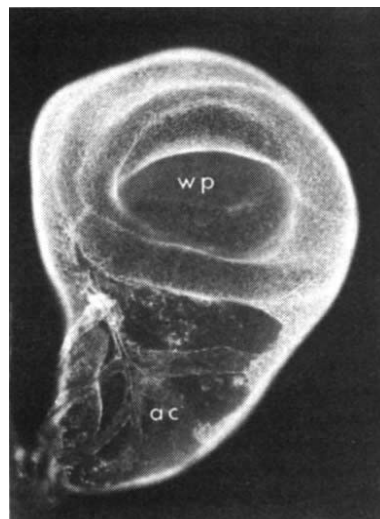


Fig. 1 Late third-instar wing imaginal disk stained with a monoclonal antibody that binds to all of the epithelial cells. The deep wing pouch (wp) in the highly folded epithelium contains the precursors of the adult wing blade, while the more peripheral regions will form proximal structures of the mesothorax¹⁸. Non-epithelial muscle precursors (adeptial cells, ac) are found over the presumptive notum; these do not stain with this antibody. In this and subsequent figures, anterior is to the left, posterior to the right.

fluorescent patches in the dorsal half¹⁶ (Fig. 2). These dorsal patches show antero-posterior asymmetries, the most noteworthy being a large patch of fluorescence in the posterior half of the central (presumptive distal) wing pouch. In *engrailed* disks, this posterior patch is absent, so that the posterior wing pouch looks very similar to the anterior half. In the most peripheral (presumptive proximal) regions, the PS2 pattern shows clear antero-posterior asymmetries in the ventral half of the disk¹⁶; these patterns are similar in both wild-type and *engrailed* disks (not shown). This finding is consistent with the observation that the cuticular derivatives of these cells show no clear mutant phenotype in *engrailed* adult flies⁸.

At the end of the second larval instar, the wing disk epithelium is a smooth sac of 2,000–3,000 cells, compared with about 50,000 cells in the highly folded mature disk. The PS2 immunofluorescence pattern is also relatively simple in the early disk, and in the ventral half displays a sharp antero-posterior difference in intensity¹⁷ (Fig. 3). This sharp difference is not seen in *engrailed* disks; the fluorescence intensity in the posterior half is greatly increased, typically giving these disks a double-anterior appearance.

The *engrailed* phenotype in late third-instar wing disks is consistent with the posterior-to-anterior transformation hypothesis, but does not exclude other possibilities. Because the observable differences in the mature disk are localized and assayed relatively late in development, it is difficult to rule out the possibility that they result from a regulative response to localized areas of cell death induced by the *engrailed* mutation, as opposed to a transformation of cellular identity. It is much more difficult, however, to explain the PS2 pattern in *engrailed* second-instar disks by mechanisms that invoke local cell death or general pattern disruption. To produce and maintain^{14,22} extensive pattern alteration via localized death and regulation would require major changes in cell proliferation patterns in the posterior compartment, and clonal analyses have not detected the predicted changes^{8,9}. Moreover, the enhanced posterior fluorescence in second-instar *engrailed* disks encompasses a large fraction of the wing primordium yet does not extend to the posterior dorsal cells, whose anterior counterparts also are

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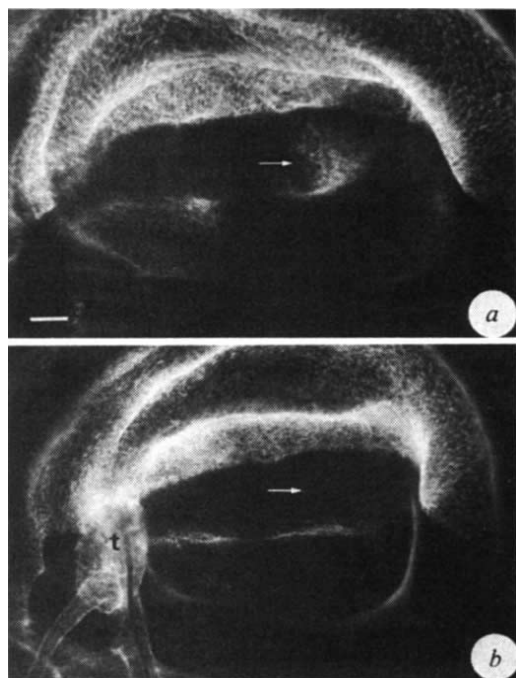


Fig. 2 PS2 immunofluorescence on wing pouch regions of late third-instar disks from wild-type (a) and *en¹/en¹* (b) larvae. Intense fluorescence is observed in all of the ventral (upper) half of the pouch. In the dorsal half, a large patch of fluorescence is observed in the posterior region of the wild-type pouch (arrow). The patch is absent from the *engrailed* disk (b). The disappearance of this patch from the distal (central) wing pouch is the only clear evidence of transformation detectable in the mature disk using the PS2 antibody. Morphologically, the posterior region of the wing pouch is typically enlarged in *engrailed* disks, making the pouch appear more rectangular. t, Trachea. Scale bar, 25 μ m.

Methods: The generation of antibodies and the immunofluorescence procedures used here have been detailed elsewhere¹⁶. Briefly, wing imaginal disks were dissected in *Drosophila* Ringer's solution and stained with a nitro blue tetrazolium solution which makes the disks opaque and lightly fixes them. Then the disks were incubated in PS2 antibody (CF.2C7), washed, incubated in fluorescein-conjugated goat anti-mouse γ -globulin (Antibodies Incorporated), washed, and fixed in formaldehyde. Although not shown, the disks were subsequently incubated in an antibody (CF.7A6) that binds to all of the epithelial cells (Fig. 1) and which was labelled with a different fluorochrome. This allows visualization of the disk morphology and also controls for possible permeability artefacts. The disks were mounted in 0.2–5% *n*-propyl gallate to reduce fluorescence bleaching²³ and viewed by epi-illumination on a Zeiss photomicroscope. Photographs were taken with Kodak Tri-X film developed at ASA 1600. The flies used were the Barton wild-type strain of *Drosophila melanogaster* and *en¹/en¹* homozygous animals. Although the fertility of *en¹/en¹* crosses is very low (probably due to *engrailed* effects in the *genitalia*¹³), homozygous larvae could be generated by mixing large numbers of *en¹* virgins and males, obtained from an *en¹/Cy0* stock.

not strongly fluorescent. This precision in the transformation argues against a mechanism involving a general disruption of pattern. Indeed, because the PS2 antigen allows one to assay such a large fraction of the wing cells at such an early developmental stage, the results from the second-instar disks provide perhaps the strongest evidence to date that mutations at the *engrailed* locus effect a transformation of cells in the posterior compartment to anterior identity.

While the transformation seen in the early wing disk, as monitored by the PS2 antibody, may be better than that seen in the wing cuticle, it still is not complete. Although the posterior

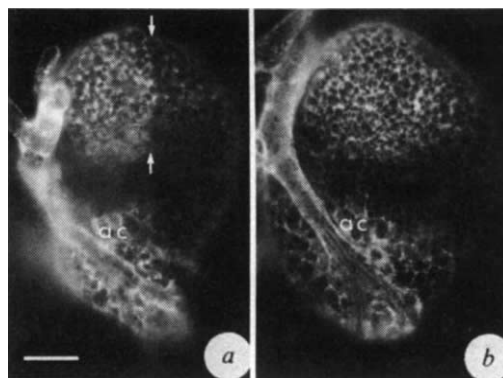


Fig. 3 PS2 immunofluorescence on late second-instar wing disks from wild-type (a) and *en¹/en¹* (b) larvae. Over much of the wild-type disk the fluorescence is more intense on the anterior (left) half of the epithelium, with a sharp boundary (arrows) separating this region from the more weakly staining posterior (right) half. In the *engrailed* disk, this boundary has disappeared, with bright immunofluorescence extending into the posterior region. There is relatively little staining in the dorsal (lower) half of the disk, except for the adepithelial cells (ac). Second-instar disks are very simple in morphology; the location of the anterior side is determined by the position of the tracheal branch. Scale bar, 25 μ m.

fluorescence is greatly increased in *engrailed* disks, it is sometimes not as intense as the anterior fluorescence. Furthermore, in both the late wing disk and its adult derivatives, proximal regions are largely unaffected. The lack of a perfect posterior-to-anterior transformation was once thought to be due to leakiness of the original *engrailed* (*en¹*) allele^{6,8,9}. However, clonal analyses have recently shown that the wing transformation induced by lethal (apparent null) alleles at the locus is no more complete than that caused by *en¹* (refs 10–12). Thus, while mutations at the *engrailed* locus can cause cells in the posterior compartment to become more anterior-like, the notion that the *engrailed* locus alone provides a direct anterior–posterior binary switch controlling the developmental regulation of cells, is probably an oversimplification. It seems likely that other genes, perhaps acting in concert with the *engrailed* gene, also are involved in specifying posterior as opposed to anterior development.

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- Garcia-Bellido, A., Ripoll, P. & Morata, G. *Dev. Biol.* **48**, 132–147 (1976).
- Steiner, E. *Wilhelm Roux Arch. dev. Biol.* **180**, 9–30 (1976).
- Struhl, G. *Dev. Biol.* **84**, 372–385 (1981).
- Morata, G. & Lawrence, P. A. *Dev. Biol.* **70**, 355–371 (1979).
- Kornberg, T. *Dev. Biol.* **86**, 363–381 (1981).
- Morata, G. & Lawrence, P. A. *Nature* **255**, 614–617 (1975).
- Morata, G., Kornberg, T. & Lawrence, P. A. *Dev. Biol.* **99**, 27–33 (1983).
- Garcia-Bellido, A. & Santamaria, P. *Genetics* **72**, 87–104 (1972).
- Lawrence, P. A. & Morata, G. *Dev. Biol.* **50**, 321–337 (1976).
- Kornberg, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1095–1099 (1981).
- Lawrence, P. A. & Struhl, G. *EMBO J.* **1**, 827–833 (1982).
- Eberlein, S. & Russell, M. A. *Dev. Biol.* **100**, 227–237 (1983).
- Epner, F. & Sanchez, L. *Dev. Biol.* **100**, 387–398 (1983).
- Szabad, J., Simpson, P. & Nothiger, R. *J. Embryol. exp. Morph.* **49**, 229–241 (1979).
- Wilcox, M., Brower, D. L. & Smith, R. J. *Cell* **25**, 159–164 (1981).
- Brower, D. L. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Brower, D. L., Piovant, M. & Reger, L. A. *Dev. Biol.* (submitted).
- Bryant, P. J. *J. exp. Zool.* **193**, 49–78 (1975).
- Ursprung, H. in *The Biology of Imaginal Disks* (eds Ursprung, H. & Nothiger, R.) 93–107 (Springer, Berlin, 1972).
- Wilcox, M. *et al. EMBO J.* (in the press).
- Sprey, Th. E., Eskens, A. A. C. & Kuhn, D. T. *Wilhelm Roux Arch. dev. Biol.* **191**, 301–308 (1982).
- Abbott, L. C., Karpen, G. H. & Schubiger, G. *Dev. Biol.* **87**, 64–75 (1981).
- Giloh, H. & Sedat, J. W. *Science* **217**, 1251–1255 (1982).

Fluorescent latex microspheres as a retrograde neuronal marker for *in vivo* and *in vitro* studies of visual cortex

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The use of retrograde axonal transport of various substances (for example, enzymes, lectins, synthetic fluorescent compounds) has yielded much information on the organization of neuronal pathways. Each type of retrograde tracer has its own set of attributes which define the scope of problems it can address¹⁻³. We describe here a new class of retrograde tracer, rhodamine-labelled fluorescent latex microspheres (0.02–0.2 μm diameter), which have distinct advantages over other available tracers for *in vivo* and *in vitro* applications. When injected into brain tissue, these microspheres show little diffusion and consequently produce small, sharply defined injection sites. Once transported back to neuronal somata, the label persists for at least 10 weeks *in vivo* and 1 yr after fixation. Microspheres have no obvious cytotoxicity or phototoxicity as assessed by intracellular recording and staining of retrogradely labelled cells in a cortical brain slice preparation. This approach was further used to visualize and compare, in cat visual cortex slices, neurones with different projection patterns, and revealed significant differences in patterns of intrinsic axons and dendrites. These properties of microspheres open new avenues for anatomical and physiological studies of identified projection neurones in slices as well as in dissociated cell cultures.

The methodology for obtaining retrograde labelling was studied by injections into the visual cortex (area 17, $n = 40$) and corpus callosum ($n = 3$) of adult rats. The first 20 rats were used to establish parameters such as the concentration and volume of microsphere suspensions, microsphere size, survival time and histological procedures. The 23 remaining rats were used to ascertain the effective injection site and to test for uptake by fibres, pathway selectivity, long-term survival effects and toxicity. Using a standardized protocol (see Fig. 1 legend), 21 of the 23 rats injected showed retrograde labelling.

Injection sites, 50–200 μm in diameter (Fig. 1a), remained similar in size at 6 h or 10 weeks post-injection. Their restricted extent probably results from the relatively large size (on a molecular scale) of the microspheres, and the binding properties of their hydrophobic surfaces (for example, their tendency to stick to cell membranes). Semi-thin sections of the injection site revealed apparently healthy tissue and normal-looking neurones; the only signs of injury were numerous microsphere-filled macrophages invading the electrode track.

Injections into area 17 resulted in retrograde labelling locally within area 17, in cortical areas outside area 17, and subcortically. We measured the extent of retrograde labelling in the lateral geniculate nucleus (LGN) to estimate the effective site of microsphere uptake. Injections into the peripheral representation of the upper visual field typically produced a cluster (0.15–0.3 mm across) of labelled LGN neurones. The part of the visual field represented by such a cluster corresponds with the area included in the cortical injection site^{4,5}; thus, the injection site boundaries delineate the effective site of uptake.

Retrograde transport was obtained after 12-h survival times, improved over 48 h and remained completely unchanged in extent or quality even after 10 weeks survival time.

The results of injections in area 17, typically including layer IV and parts of layer II/III, were compared with published horseradish peroxidase (HRP) labelling (HRP is transported by virtually all neural pathways³). The known connections of rat area 17⁶ were all labelled, suggesting that microsphere uptake and transport is not selective for specific neuronal pathways. These injections also labelled a previously undescribed set of intrinsic connections within area 17. When the injection site was about 150 μm in diameter, labelling was seen immediately surrounding the injection site; in layer V, retrogradely labelled cells were spread over an area about five times the diameter of the injection site. Upper layer VI was generally free of label. In sharp contrast to this, cells in lower layer VI were found in an area about 10 times the diameter of the injection site (Fig. 1b). The lateral extent of label in layer V and lower layer VI indicates that these neurones have widespread lateral axonal connections (A.B. and L.C.K., unpublished observations), consistent with the morphology of some layer V and VI cells in cat area 17⁷. Label in the extrastriate visual areas 18a and 18b included layers II–VI. Subcortical label was observed in the ipsilateral LGN (Fig. 1d) and in the lateral posterior thalamic nucleus. Microspheres are also transported by broken axons. Injections into the corpus callosum resulted in many densely labelled pyramidal cells in layers II/III, V and VI throughout the cortex (Fig. 1e). When, in one experiment, instead of injecting the corpus callosum, 4 μl of microspheres were placed on top of it, no retrograde labelling was produced. This suggests that although severed fibres take up and transport microspheres, unbroken fibres of passage do not.

In all labelled neurones, label, confined to the cytoplasm, filled the soma and the proximal dendrites (Fig. 1c, d, f) with a granular fluorescence, leaving nuclei unlabelled.

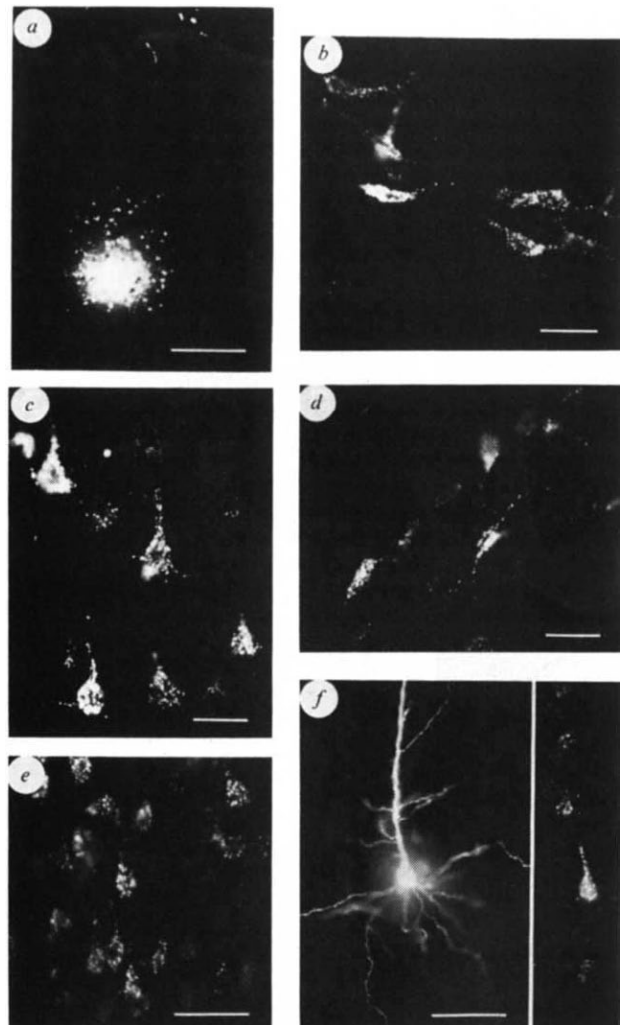
All structures which contained retrogradely labelled cell bodies are reciprocally connected to area 17^{6,8}, but close examination failed to reveal any evidence of anterograde labelling. The same negative result was found in the superior colliculus, a known target of layer V pyramidal cells⁹. Thus, at the level of the light microscope, microspheres are an exclusively retrograde tracer.

Retrograde labelling of cat area 17 pyramidal cells, combined with an *in vitro* brain slice preparation, was used to study the morphology and distribution of the dendritic and axonal arbors of LGN¹⁰ and claustrum-projecting¹¹ cells co-localized within layer VI. Three days after microsphere injections into either the LGN or claustrum, slices of area 17 were prepared and microsphere-labelled cells were intracellularly injected with Lucifer yellow (Fig. 1 legend). Fifty LGN-projecting cells (in 4 cats) and 30 claustrum projecting cells (3 cats) were analysed. All the double-labelled neurones showed normal resting, synaptic and action potentials whose size (45 ± 5 mV compared with 42 ± 5 mV) and time course were indistinguishable from those of unlabelled neurones. Morphologically, the double-labelled cells showed no evidence of degeneration in axons, dendrites or dendritic spines (Fig. 1f). Thus, microspheres did not have obvious toxic effects, and intermittent illumination with rhodamine excitation wavelengths *in vitro* for several minutes did not seem harmful. Comparison of these two efferent projection classes revealed striking differences in the laminar distribution of dendritic and intrinsic axonal arbors (Fig. 2). Claustrum-projecting cells had roughly half as many basal dendritic arms (3–5) as did LGN-projecting cells (6–8). The apical dendrites of claustrum-projecting cells reach to layer I with branches in layers VI and V only; LGN-projecting cells branched in layers VI, V and IV, but did not reach above layer III. Finally, all claustrum-projecting cells had fine, horizontally directed axonal collaterals that extended for up to 1 mm within layer VI. In contrast, LGN-projecting neurones had very few axon collaterals within layer VI, but exhibited thick, ascending processes which terminated in layer IV. These two classes therefore not

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Fig. 1 Retrograde labelling by fluorescent rhodamine latex microspheres. *a*, Injection site, 100 μm diameter, produced by injection of 0.02–0.05 μl of microsphere suspension into layer IV of rat visual cortex. *b*, A patch of cells in layer VI in area 17, at the grey/white matter border, labelled after an injection $\sim 400 \mu\text{m}$ away that included layers III and IV. *c*, Labelling of layer V pyramidal cells in area 18b after area 17 injection. Note labelling of apical and basal dendrites, low background and distinctive granular appearance of label. *d*, Lateral geniculate nucleus labelling after an area 17 injection. *e*, Dense retrograde labelling of pyramidal cells in layers II/III after corpus callosum injection, indicating transport by broken axons. *f*, LGN projecting neurone in layer VI of area 17 from a cat *in vitro* cortical brain slice preparation, retrogradely labelled with microspheres and intracellularly injected with Lucifer yellow; the soma, dendrites and dendritic spines appear normal. Inset: Single exposure micrograph of microsphere-labelled soma. Scale bars: *a*, 100 μm ; *b*, *c*, *d*, *f*, 20 μm ; *e*, 50 μm .

Methods: An aqueous suspension of rhodamine-labelled acrylic microspheres (0.02–0.2 μm in diameter) was obtained from Dr Boyse Burge, International Diagnostics Technology (Santa Clara, California). Anaesthetized rats were injected with 0.02–0.1 μl of microsphere suspension using a glass pipette with 30–50 μm tip diameter. After survival times of 24–72 h, rats were re-anaesthetized and perfused with phosphate buffer (0.1 M) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) (glutaraldehyde can be used as a fixative, although the resulting higher background may make microsphere labelling more difficult to discern). After the brains had been sunk in 30% sucrose in buffer, 30 μm sections were cut on a freezing microtome, collected in phosphate buffer, mounted on gelatinized slides and either air dried or inspected wet. Brief dehydration (30 s, 100% ethanol, after air drying), followed by clearing in xylenes (60 s) and mounting in Fluoromount (Gurr) or Krystalon (Harleco) often significantly improved the visibility of label. However, longer exposures to ethanol, and in particular to xylenes, causes serious deterioration of the fluorescence. Observations were made using a rhodamine filter combination (Zeiss filters, exciter BP 510–560, beam splitter FT 580, barrier LP 590) on an epifluorescence-equipped microscope. Labelled neurones are readily visible in sections prepared over 1 yr previously, stored either dry in the dark or unmounted in 4% formaldehyde in phosphate buffer at 4°C. Cat cortical slices (400 μm thick, coronal sections) were prepared and maintained using essentially standard hippocampal slice techniques²⁴. For intracellular recording and staining, slices were transferred to a chamber on the stage of an epifluorescence-equipped compound microscope. Microsphere-labelled cell bodies can be seen in living tissue even at low magnifications ($\times 100$ or $\times 160$). Cells in layer VI were impaled with micropipettes filled with 20% Lucifer yellow (Aldrich) in 0.1 M LiCl (final resistance 100–150 M Ω). Neurones with stable resting potentials and action potentials of at least 35 mV amplitude were filled with dye by passing hyperpolarizing current pulses (3 nA, 200 ms, 4 Hz, 1–10 min). Slices were fixed in phosphate-buffered 10% formalin for at least 2 h and processed as described for brain sections (except that the sections were 60 μm thick).



only presumably receive different afferent inputs via different dendritic patterns, but their non-overlapping intrinsic axon patterns indicate participation in distinct circuits within area 17, and may be involved in the extraction of feedback (to the LGN) and feedforward (to the claustrum) signals.

The mechanism(s) by which microspheres are taken up and transported are unknown. However, not all latex microspheres are retrogradely transported. Experiments with coumarin-labelled carboxy-activated (0.05 μm diameter) or plain polystyrene (0.1 μm diameter) microspheres (Polysciences) produced no observable labelling; neither did larger-diameter microspheres ($>0.2 \mu\text{m}$) of the type described here. Thus, both the size and surface properties¹² may play a part. Latex microspheres can bind many proteins, including antibodies, non-covalently (which is the basis of most latex particle agglutination tests¹³); possibly, microspheres below a certain size can enter synaptic clefts, and interactions between molecules on synaptic surfaces and hydrophobic groups on the microsphere's surface could trigger an endocytotic event¹⁴ (endocytosis of latex particles by many cells, including amoebae¹⁵ and platelets¹⁶, is well known). Endocytotic vesicles containing microspheres may then be transported by fast retrograde transport¹⁷. The uptake by broken fibres may be explained by the formation of growth cones at the proximal ends of severed axons¹⁸, followed by internalization and subsequent retrograde transport of micro-

spheres bound to membrane components¹⁹. Microspheres are probably transported packed in vesicles, because naked latex microspheres with similar negative charge move only in the anterograde direction when injected directly into axons¹². This, however, does not rule out vesicle-bound anterograde transport of microspheres, which may have remained undetected²⁰.

The resistance of these rhodamine-labelled microspheres to fading under illumination, and their non-phototoxicity within cells are probably related, as the relationship between bleaching time and killing time is usually linear²¹. Both photodynamic damage and bleaching depend on the presence of molecular oxygen²² and probably result from the formation of free radicals. Hydrophobic polymers, by restricting the access of molecular oxygen to the rhodamine dye in the interior of the microspheres, could greatly attenuate the formation and resultant phototoxicity of destructive free radicals.

These microspheres exhibit a variety of characteristics available in very few tracers. The highly restricted injection sites, the compatibility with all known retrograde and anterograde tracers and with immunocytochemical procedures (A.B., R. W. Baughman and L.C.K., in preparation) makes microspheres particularly useful for the study of local circuitry within or between brain areas. The exceptionally long stability of this label makes it attractive for developmental studies on the reshaping of patterns of axonal connections and as a marker in transplantation

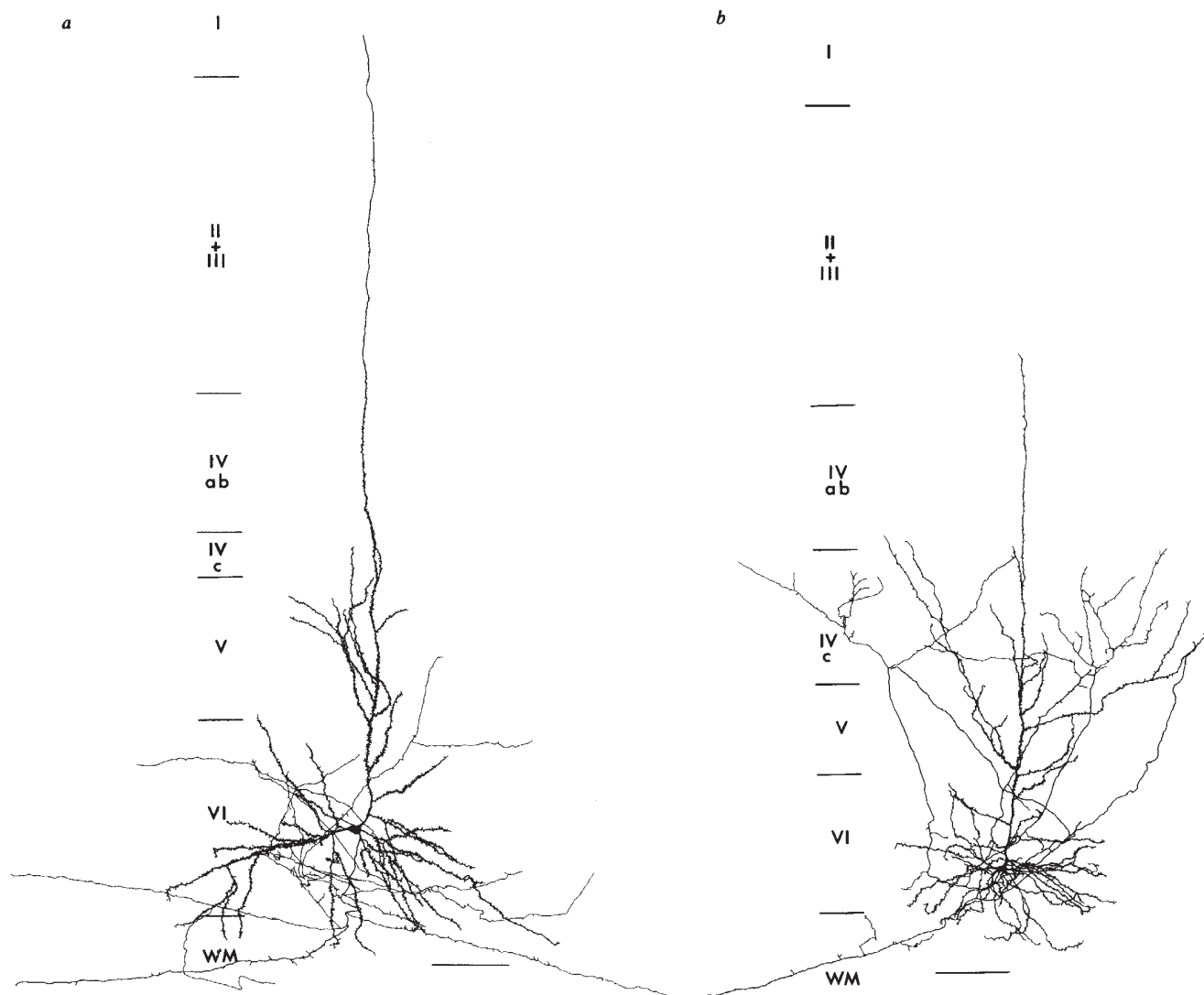


Fig. 2 Camera lucida drawings of claustrum (*a*) and lateral geniculate nucleus (*b*) projecting neurones in cat area 17, obtained as described in Fig. 1 legend and in the text. The numerals refer to laminar boundaries. Claustrum-projecting cells had a thick, often asymmetrical basal dendrite, in contrast with the compact, symmetrical arbor of LGN-projecting cells. The apical dendritic side branches of these two projection classes terminated within different laminae. Most conspicuously, the claustrum-projecting cells (*a*) had thin, horizontally directed intrinsic axonal collaterals, which remained within layer VI. The LGN-projecting cells (*b*) had few horizontal collaterals in VI, but sent thick ascending collaterals to layer IV, in which they terminated.

studies. The most outstanding property of this tracer, however, is that the viability of neurones is unaffected by transported microspheres. Fluorescent microspheres can therefore be used for *in vitro* studies of identified projection neurones and their role in cortical circuits. Preliminary experiments indicate that microsphere labelling can also be used as a marker for dissociated neurones in culture isolated in a fluorescence-activated cell sorter (J. E. Huettner and R. W. Baughman, personal communication).

Although numerous approaches exist for identifying neuronal cell classes, very few of those presently available can be used to study living cells. Recently, a specific cell-surface marker has been used to study the physiology of identified neurones in culture²³. The approach described here may provide an attractive alternative for the visualization and physiological study of projection neurones.

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1. Jones, E. G. & Hartman, B. K. *A. Rev. Neurosci.* **1**, 215–296 (1978).
2. Aschoff, A. & Hollander, H. *J. Neurosci. Meth.* **6**, 179–197 (1982).
3. Mesulam, M.-M. (ed.) *Tracing Neural Connections with Horseradish Peroxidase* (Wiley-Interscience, New York, 1982).
4. Montero, V. M., Brugge, J. F. & Beitel, R. E. *J. Neurophysiol.* **31**, 221–236 (1968).
5. Montero, V. M., Rojas, A. & Torrealba, F. *Brain Res.* **53**, 197–201 (1979).
6. Miller, M. W. & Vogt, B. A. *J. comp. Neurol.* (in the press).
7. Gilbert, C. D. & Wiesel, T. N. *J. Neurosci.* **3**, 1116–1133 (1983).
8. Lent, R. *J. comp. Neurol.* **206**, 227–242 (1982).
9. Sefton, A. J., Mackay-Sim, A., Baur, L. A. & Cottee, L. J. *Brain Res.* **215**, 1–13 (1981).
10. Gilbert, C. D. & Kelly, J. P. *J. comp. Neurol.* **163**, 81–106 (1975).
11. LeVay, S. & Sherk, H. *J. Neurosci.* **1**, 956–980 (1981).
12. Adams, R. J. & Bray, D. *Nature* **303**, 718–720 (1983).
13. Milgrom, F. & Golstein, R. *Vox Sang.* **7**, 86–88 (1962).
14. Gonatas, N. K., Kim, S. U., Stieber, A. & Avrameas, S. *J. Cell Biol.* **73**, 1–13 (1972).
15. Korn, E. D. & Weisman, R. A. *J. Cell Biol.* **34**, 219–227 (1967).
16. Movat, H. Z., Weiser, W. J., Glynn, M. F. & Mustard, J. F. *J. Cell Biol.* **27**, 531–543 (1965).
17. Schwab, M. E. & Thoenen, H. *J. Cell Biol.* **77**, 1–13 (1978).
18. Aguayo, A. J., Benfy, M. & David, S. *Birth Defects* **19**, 327–340 (1983).
19. Carbonetto, S. & Argon, Y. *Dev. Biol.* **80**, 364–378 (1980).
20. Brady, S. T. & Lasek, R. J. *Science* **218**, 1127–1131 (1982).
21. Gupta, R. K. *et al. J. Membrane Biol.* **58**, 123–127 (1981).
22. Salzberg, B. M. in *Current Methods in Cellular Neurobiology* Vol. 3 (eds Barker, J. C. & McKelvig, J. F.) 139–187 (Wiley-Interscience, New York, 1983).
23. Kettenmann, H., Weinrich, M. & Schachner, M. *Neurosci. Lett.* **41**, 85–90 (1983).
24. Dingleline, R., Dodd, J. & Kelly, J. S. *J. Neurosci. Meth.* **2**, 323–362 (1980).

A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones

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Calcium channels in excitable membranes are essential for many cellular functions. Recent analyses of the burst-firing mode of some vertebrate neurones¹⁻³ suggest that changes in their functional state are controlled by a Ca conductance that is largely inactivated at resting membrane potentials (-50 to -60 mV), but becomes activated following a conditioning hyperpolarization of the cell membrane. Here, using chick and rat sensory neurones, we present evidence for a new type of Ca channel with time- and voltage-dependent properties which is probably responsible for the inactivation behaviour of the Ca conductance. At membrane potentials between -50 and $+10$ mV, openings of this channel last 3–6 ms and tend to occur in rapid succession. Inactivation of this channel is indicated by prolonged and eventually complete closures brought about by long-lasting depolarizing voltage steps. This channel coexists in isolated membrane patches with the more common Ca channel⁴ which is less sensitive to changes in holding potential and shows a considerably shorter average life time and smaller currents.

Single-channel currents were recorded from excised, outside-out membrane patches using cultured dorsal root ganglion cells from 10-day-old chick embryos and 18-day rat embryos⁵. This method⁶⁻⁸ permits the membrane potential and intracellular ionic content to be controlled directly. The membrane patches lasted an average of 5–20 min, during which a deterioration of Ca channel activity was rarely observed under the precaution of high-resistance seals, sufficiently negative holding potentials (< -80 mV) and low intracellular Ca concentrations. Before the patch was made⁸, whole-cell currents were investigated up to the limits (1 nA) of current strengths permitted by the 10-G Ω feedback resistor of the current-voltage converter. Recordings were made at 0–3 kHz bandwidth, with the data filtered at 1 kHz by zero phase digital filtering. Linear components of leakage and capacitance were subtracted. Control and test records of 200–500 ms duration were performed at intervals greater than 10 s to avoid cumulative inactivation.

Figure 1 shows recordings of pharmacologically isolated inward Ca currents. Both whole-cell and single channel currents were blocked by millimolar additions of Cd^{2+} , Co^{2+} or Ni^{2+} to the bath. Stepwise depolarizations from holding potentials between -80 and -110 mV⁹ produced inward Ca currents at -50 to -40 mV. They showed time- and voltage-dependent inactivation and their peak amplitude increased gradually with increasing membrane depolarization. Less negative holding potentials strongly reduced these inward currents (Fig. 1b), a feature which is not encountered in Ca currents from other cell preparations¹⁰⁻¹². A second, larger rise of peak inward current occurred between -15 and 0 mV. Only this part of the current-voltage relationship corresponds to the behaviour of Ca currents usually encountered (for a review see ref. 13). These currents showed little time-dependent inactivation in the presence of intracellular EGTA and little response to a reduction in the negativity of the holding potential (Fig. 1b).

The current reduction was almost completely restricted to the transient peak. It is interesting that the time to peak of the overall Ca current was shortest between membrane potentials of -30 and -10 mV (Fig. 1a), at which non-uniform channel activation was already detectable.

Figure 1c shows single-channel recordings during voltage step changes to -40 mV. With a holding potential of -60 mV (Fig. 1c, left), channel opening was largely absent, but it occurred in

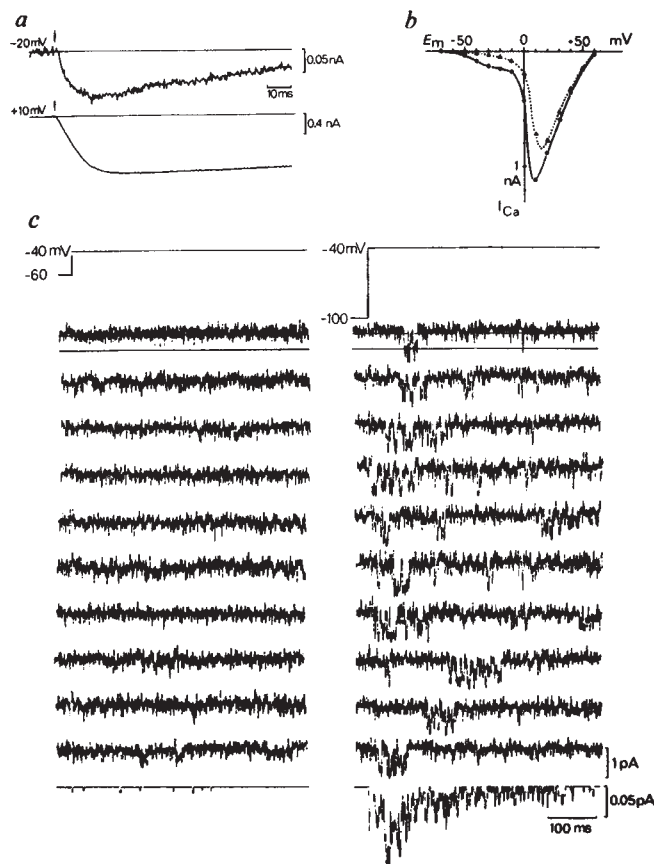
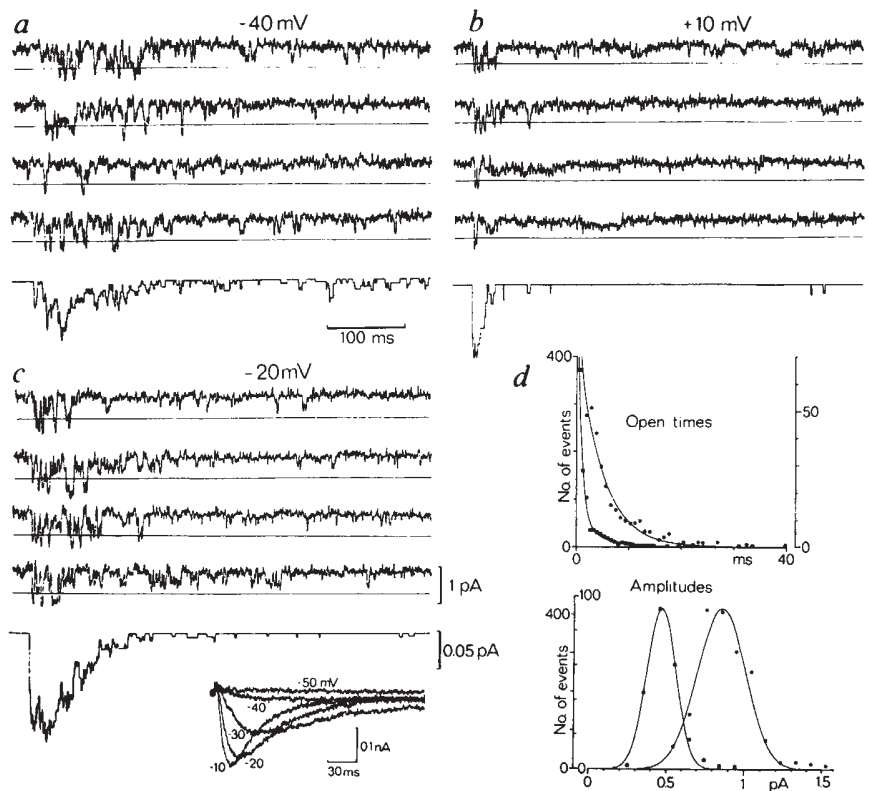


Fig. 1 Activation of whole-cell and unitary Ca currents of the chick dorsal root ganglion cell membrane during depolarizing voltage steps from strongly negative holding potentials. **a**, Appearance of an inactivating Ca current of the whole cell during a step to -20 mV from -90 mV membrane potential (E_m) (upper trace). Both turn-on and subsequent inactivation of this current are considerably faster than those of the current during a stronger depolarizing step ($+10$ mV, lower trace) from the same holding potential. **b**, Peak current-voltage relationship of whole-cell inward current from a holding potential of -90 mV (continuous line) and -60 mV (dotted line). **c**, Unitary Ca currents of an outside-out patch appear (right) during successive depolarizing steps to a low membrane potential (-40 mV) after the holding potential had been increased to -100 mV from an initial -60 mV (left). Lines in the upper current traces give the detection level for computing openings with the level set at 3.5 times background r.m.s. from zero baseline. The r.m.s. value was determined from event-free intervals and compared with that of the recordings with the inactivated channel (left). Amplitudes were computed by averaging between crossings of level, with opening durations determined at half amplitudes. A second level near baseline at a similar distance from the average amplitude served to define closures. Sample averages of computed events are given in the bottom traces. Each sample contained 21 recordings. Temperature, 12°C . The pipette solution contained (in mM): 100 CsCl, 16 tetraethylammonium-Cl, 1.6 MgCl_2 , 0.2 CaCl_2 , 5 EGTA, 7 glucose, 10 HEPES-NaOH at pH 7.2. The bath composition was (in mM): 120 choline-Cl, 20 CaCl_2 , 2 MgCl_2 , 10 HEPES-CsOH (pH 7.2) plus $3\text{ }\mu\text{M}$ tetrodotoxin. Identical results were obtained when choline was replaced by Na. In this preparation, $3\text{ }\mu\text{M}$ tetrodotoxin has previously been observed to block fully the inward Na current in external solutions containing high Na and low Ca^{2+} .

the form of discrete inward-going unitary events at the same test potential when the holding potential was set at -100 mV (Fig. 1c, right). Openings were frequently interrupted by short-lasting closures and thus assumed the appearance of bursts. The probability of the occurrence of opening was higher shortly after the onset of the step to -40 mV than at later times, indicating a time-dependent inactivation. Mean amplitude and open time were not affected and inactivation was equally strong in samples

Fig. 2 Ca channel currents in an outside-out patch from a rat dorsal root ganglion cell, recorded at the potential indicated, from a holding of -90 mV. Lines in the first four recordings represent the detection level for opening. Events were determined as in Fig. 1 and were averaged (last trace at each section) over 29 (a), 27 (b) and 10 (c) trials. Large- and small-amplitude events appear in the recordings, with the larger unitary currents occurring predominantly in the initial period of the potential step. The detection level for the large-amplitude events was by $2 \times$ s.d. set beyond the mean amplitude of the smaller unitary currents. The latter events were collected after inactivating the large-amplitude channel. Inset in b, whole-cell Ca currents recorded from a rat dorsal root ganglion cell at the potentials indicated. Holding potential, -90 mV. Note the voltage-dependent time courses of activation and inactivation. d, Amplitude and open time histograms of unitary Ca currents at -40 mV membrane potential. The amplitude distributions were fitted by gaussian curves. The left-hand side distribution (left scale) has a maximum of 0.45 and a s.d. of 0.085 pA. It contains some large-amplitude events that escaped inactivation and thus contribute to the overlapping of the two distributions. The large-amplitude distribution (right scale) has a maximum of 0.86 and a s.d. of 0.15 pA. It is wider because it incorporates a fraction consisting of simultaneous openings of large and small amplitude. Exponential functions were fitted to the open time histograms, with resulting time constants of 0.72 and 4.67 ms for the small and large unit, respectively. The considerable differences between these values strengthen the conclusion that two different populations of Ca channels were separated rather than two levels of current resulting from superpositions of small-amplitude events.



with high and low initial frequency of openings. The sample averages of the channel currents (Fig. 1c, right, bottom trace) showed a near complete return to zero within 400 ms after attaining a peak within ~ 40 ms. The mean open times of these channels were calculated to be 4.2 ± 0.8 ms (mean and s.d., $n = 15$), which is three- to seven-fold greater than the mean open time of previously described Ca channels in this and other preparations (ref. 4, see also Fig. 2d). The mean of the amplitude histograms at -40 mV was 0.89 pA, which decreased to 0.51 pA at 0 mV, giving a conductance slope of 9.5 pS within this range of potential. These values are 30–40% greater than those of the incompletely inactivating Ca channel^{4,14}.

Increasing the size of the depolarizing steps shortened the average time to first opening while speeding up channel inactivation (Fig. 2). This can be seen from the sample averages of the currents taken at different potentials (bottom traces of Fig. 2a–c). Thus, at -40 mV, the time to peak (t_p) was about 35 ms and inactivation occurred with a time constant (τ_h) of 55 ms, while at $+10$ mV t_p and τ_h decreased to 5 and 9 ms, respectively. Evaluation of the voltage dependence of inactivation in eight membrane patches, using current averages taken at two or more potentials (range -50 to $+10$ mV), showed an e-fold reduction of τ_h for a potential change of 22 ± 3 mV.

Times to peak and time constants of inactivation from the sample averages of the unitary currents agreed well with those of whole-cell currents (Fig. 2b, inset), except for a shift of -10 to -15 mV in the voltage dependence of the parameters of activation and inactivation. This is similar to previous observations^{10,15} on the Na channel using the same technique. The whole-cell currents showed less complete steady-state inactivation; at -20 mV, these currents settled at a level which was about 15% of the peak current. This is probably due to a contribution of the usually observed Ca channel which is known to become inactivated slowly and incompletely^{10,13} in the present conditions of internal Ca buffering.

The fast-inactivating, low potential-activated channel and the commonly observed Ca channel appeared together in most, but not all, patches. The failure of occurrence of one or the other type of events suggests different kinds of channels rather than different states of the same channel. In this respect, it is noteworthy that we detected no Ca channels with properties similar to those described here in similarly treated cultured sympathetic neurones or in molluscan neurones¹². On the other hand, Hagiwara *et al.*¹⁶ found two separate Ca conductance mechanisms (types I and II) in the egg cell membrane of the starfish. Type I showed time- and voltage-dependent inactivation similar to that described here, but its activation was markedly affected by external sodium. The phylogenetic relationship between this Ca system and the kinetically similar Na system is unclear¹⁶, as is the function of this Ca channel, apart from a possible involvement in the cyclical firing pattern of several central neurones^{1–3}.

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1. Llinas, R. & Yarom, Y. *J. Physiol., Lond.* **315**, 569–584 (1981).
2. Llinas, R. & Jahnsen, H. *Nature* **297**, 406–408 (1982).
3. Jahnsen, H. & Llinas, R. *J. Physiol., Lond.* **349**, 205–226 (1984).
4. Brown, A. M., Camerer, H., Kunze, D. L. & Lux, H. D. *Nature* **299**, 156–158 (1982).
5. Barde, Y. A., Edgar, D. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1199–1203 (1980).
6. Horn, R. & Patlak, J. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6930–6934 (1980).
7. Hamill, O. P. & Sakmann, B. *J. Physiol., Lond.* **312**, 41P–42P (1981).
8. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, E. F. *J. Physiol. Arch. ges. Physiol.* **391**, 85–100 (1981).
9. Carbone, E. & Lux, H. D. *Biophys. J.* (in the press).
10. Fenwick, E. M., Marty, A. & Neher, E. *J. Physiol., Lond.* **331**, 599–635 (1982).
11. Adams, D. J. & Gage, P. W. *J. Physiol., Lond.* **291**, 467–481 (1979).
12. Lux, H. D. & Brown, A. M. *J. gen. Physiol.* **83**, 727–750 (1984).
13. Hagiwara, S. & Byerly, L. A. *Rev. Neurosci.* **4**, 69–125 (1981).
14. Lux, H. D. in *Single-Channel Recording* (eds Sakmann, B. & Neher, E.) 437–449 (Plenum, New York, 1983).
15. Nagy, K., Kiss, T. & Hof, D. *Pflügers Arch. ges. Physiol.* **399**, 302–308 (1983).
16. Hagiwara, S., Ozawa, S. & Sand, O. J. *gen. Physiol.* **65**, 617–644 (1975).

DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1

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The neurotransmitter dopamine has been demonstrated by biochemical¹, histochemical² and immunocytochemical^{3,4} techniques to be unevenly distributed in the mammalian central nervous system. DARPP-32 (dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32,000) is a neuronal phosphoprotein that displays a regional distribution in the mammalian brain very similar to that of dopamine-containing nerve terminals, being highly concentrated in the basal ganglia⁵⁻⁸. The state of phosphorylation of DARPP-32 can be regulated by dopamine and by cyclic AMP in intact nerve cells^{5,6}, suggesting a role for this phosphoprotein in mediating certain of the effects of dopamine on dopaminergic cells. The observation that many of the physical and chemical properties of purified DARPP-32⁹ resemble those of phosphatase inhibitor-1 (inhibitor-1)^{10,11}, a widely distributed inhibitor of protein phosphatase¹², suggests that DARPP-32 might also function as a phosphatase inhibitor. We report here that DARPP-32 inhibits protein phosphatase-1 at nanomolar concentrations. Moreover, like inhibitor-1¹⁰, DARPP-32 is effective as an inhibitor in its phosphorylated but not its dephosphorylated form. Thus, the basal ganglia of mammalian brain contain a region-specific neuronal phosphoprotein that is a protein phosphatase inhibitor.

The protein phosphatase activities involved in the dephosphorylation of most of the known phosphorylated proteins can be accounted for by four enzymes. These enzymes are grouped into two classes—type 1 protein phosphatase (protein phosphatase-1) and type 2 protein phosphatases (protein phosphatase-2A, -2B and -2C)¹²⁻¹⁴—according to their specificity towards model substrates and their sensitivity to inhibitors. Thus, type 1 protein phosphatase selectively dephosphorylates the β -subunit of phosphorylase kinase and is inhibited by nanomolar concentrations of inhibitor-1 or inhibitor-2, while type 2 protein phosphatases selectively dephosphorylate the α -subunit of phosphorylase kinase and are insensitive to these inhibitors¹³.

As Table 1 shows, both phospho-DARPP-32 and phospho-inhibitor-1 inhibited protein phosphatase-1 activity by >90% at a concentration of 10^{-8} M. This inhibition was observed whether phosphorylase *a* (Table 1) or phosphorylase kinase (data not shown) was used as substrate. The effect of both inhibitors was specific for protein phosphatase-1, as neither inhibited protein phosphatase-2A (Table 1), -2B or -2C (data not shown).

Figure 1 compares the effects of various concentrations of phospho-DARPP-32, dephospho-DARPP-32 and phospho-inhibitor-1 on protein phosphatase-1 activity. The EC_{50} for phospho-DARPP-32 was $\sim 10^{-9}$ M and that for phospho-inhibitor-1 was $\sim 0.7 \times 10^{-9}$ M. In contrast, dephospho-DARPP-32 was ineffective as an inhibitor up to 5×10^{-8} M (Fig. 1). Thus, DARPP-32 resembles inhibitor-1 in being active as an inhibitor of protein phosphatase-1 only in its phosphorylated form¹⁰. A kinetic analysis of the interaction of phospho-DARPP-32 and phospho-inhibitor-1 with protein phosphatase-1, using phosphorylase *a* as substrate and 0.1 U ml^{-1} of protein phosphatase-1, indicated a mixed mechanism of inhibition with respective

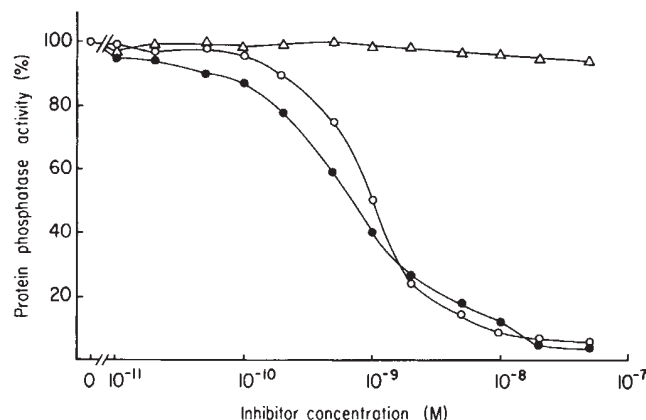


Fig. 1 Inhibition of protein phosphatase-1 by various concentrations of phospho-DARPP-32 (○), dephospho-DARPP-32 (△) and phospho-inhibitor-1 (●). The activity of protein phosphatase-1 was determined by the assay described in Table 1 legend using 1.0 U ml^{-1} of protein phosphatase-1. Protein concentrations were determined using spectrophotometrically standardized bovine serum albumin³². A molecular mass of 24,000, determined by high-speed sedimentation equilibrium centrifugation (unpublished result), was used for DARPP-32, and of 19,000, determined by amino acid sequencing³³, for inhibitor-1. Phospho-DARPP-32 and phospho-inhibitor-1 each contained 1.0 mol phosphate per mol protein (see Table 1).

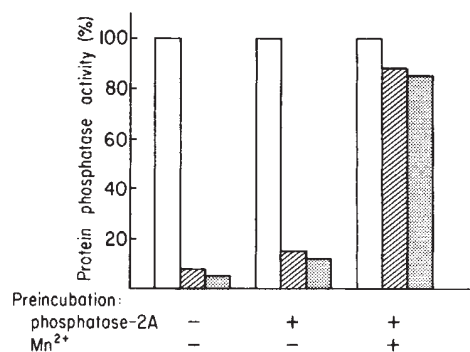


Fig. 2 Dephosphorylation of phospho-DARPP-32 and phospho-inhibitor-1 abolishes their inhibitory activity. Open bar, no addition; hatched bar, 10^{-8} M DARPP-32; stippled bar, 10^{-8} M inhibitor-1. Phospho-DARPP-32 and phospho-inhibitor-1, each at 10^{-6} M and each containing 1 mol phosphate per mol protein, were preincubated at 30°C for 2 h in the absence or presence of 5.0 U ml^{-1} of phosphatase-2A and 1 mM MnCl_2 , as indicated. The reaction mixture was heated at 95°C for 10 min to inactivate the phosphatase, and centrifuged at $2,000g$ for 5 min to remove denatured protein. The supernatants, which contained the dephosphorylated DARPP-32 and inhibitor-1, were then tested for their ability to inhibit protein phosphatase-1 using the assay described in Table 1 legend.

K_i values for the two of $\sim 0.5\text{--}2.2 \times 10^{-9}$ M (data not shown) and $1.5\text{--}7.5 \times 10^{-9}$ M (ref. 15).

The dephosphorylation of phospho-DARPP-32 by the various purified protein phosphatase preparations was compared with that of other phosphoprotein substrates (Table 2). Protein phosphatase-2A, -2B and -2C were able to dephosphorylate DARPP-32 and inhibitor-1 at significant rates, protein phosphatase-2B being particularly effective. The catalytic subunit of protein phosphatase-1 used in the present study showed little or no activity towards DARPP-32 or inhibitor-1. Note, however, that the ability of protein phosphatase-1 to dephosphorylate inhibitor-1 is labile and varies considerably between preparations^{11,13,15,16}.

The ability of phospho-DARPP-32 and phospho-inhibitor-1 to inhibit protein phosphatase-1 was abolished when they were

dephosphorylated. Preincubation of either inhibitor with protein phosphatase-2A plus Mn^{2+} resulted in a loss of inhibitory activity (Fig. 2), whereas preincubation with protein phosphatase-2A alone (Fig. 2) or with Mn^{2+} alone (data not shown) had no effect. Thus, both inhibitors can be phosphorylated and activated by cyclic AMP-dependent protein kinase and can be dephosphorylated and inactivated by type 2 protein phosphatases.

Evidence that although inhibitor-1 and DARPP-32 share many properties, they are distinct gene products and one is not

Table 1 Inhibition of protein phosphatase activity by phospho-DARPP-32 and phospho-inhibitor-1

Addition	Relative activity	
	Phosphatase-1	Phosphatase-2A
None	100 $\pm$ 3	100 $\pm$ 3
Phospho-DARPP-32 (10^{-8} M)	9 $\pm$ 2	106 $\pm$ 4
Phospho-inhibitor-1 (10^{-8} M)	6 $\pm$ 2	103 $\pm$ 3

Dephospho-DARPP-32 was purified to homogeneity from bovine caudate nucleus. Phospho-DARPP-32 was prepared from dephospho-DARPP-32 using cyclic AMP-dependent protein kinase⁹, and separated from residual ATP by chromatography on Polyanion SI (Pharmacia) anion-exchange resin. Inhibitor-1 was purified from rabbit skeletal muscle and phosphorylated using cyclic AMP-dependent protein kinase¹¹. Phospho-inhibitor-1 was further purified and separated from dephospho-inhibitor-1 and residual ATP by chromatography on DEAE-cellulose¹¹. Both phospho-DARPP-32 and phospho-inhibitor-1 contained 1 mol phosphate per mol protein based on their incorporation of ^{32}P -phosphate from [γ - ^{32}P]ATP. This stoichiometry has been confirmed by sequence analysis of phosphopeptides. The catalytic subunits of protein phosphatase-1^{28,29}, protein phosphatase-2A and ^{32}P -labelled phosphorylase *a* (100 c.p.m. μ mol⁻¹)¹⁶ were prepared from rabbit skeletal muscle. Protein phosphatase-1 and -2A were assayed by determining the release of ^{32}P -phosphate from ^{32}P -labelled phosphorylase *a*³⁰. The reactions were carried out in a volume of 60 μ l containing (final concentration) 50 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, 15 mM 2-mercaptoethanol, 5 mM caffeine, 0.6 mg ml⁻¹ bovine serum albumin, 1.0 mg ml⁻¹ ^{32}P -labelled phosphorylase *a* and 1.0 U ml⁻¹ of the indicated protein phosphatase. One unit of phosphatase activity was that amount of enzyme which catalysed the release of 1.0 nmol phosphate per min from phosphorylase *a*. After preincubation for 10 min at 30 °C, reactions were initiated with ^{32}P -labelled phosphorylase *a*, carried out for 10 min at 30 °C, terminated by the addition of 200 μ l of 20% (w/v) trichloroacetic acid, and the trichloroacetic acid-soluble radioactivity was determined by liquid scintillation spectrometry. Results represent the mean of three independent determinations $\pm$ s.e.m.

Table 2 Dephosphorylation of phospho-DARPP-32 and phospho-inhibitor-1 by various protein phosphatases

^{32}P -substrate (μ M)	Relative activity (%)			
	PrP-1	PrP-2A	PrP-2B	PrP-2C
Phosphorylase kinase (2.4)	100(β)	100(α)	100(α)	100(α)
Phosphorylase <i>a</i> (10)	33	25	1	2
DARPP-32 (2)	2	49	155	15
Inhibitor-1 (2)	2	46	162	29

Phosphorylase *a*, DARPP-32, inhibitor-1, protein phosphatase-1 and protein phosphatase-2A were prepared as described in Table 1 legend. Phosphorylase kinase was purified and phosphorylated as described previously¹⁶. Protein phosphatase-2B was partially purified from rabbit skeletal muscle²⁷ and assayed in the presence of 10 μ M calmodulin. Protein phosphatase-2C was partially purified from rabbit skeletal muscle by the method of Ingebritsen *et al.*³¹. All protein phosphatase preparations were free of other known phosphatases. Assays were performed as described in Table 1 legend except for the substitution of the indicated ^{32}P -labelled protein substrate and protein phosphatase (PrP), and the inclusion of 1.0 mM $MnCl_2$. Protein phosphatases were diluted so that $\leq 20\%$ of the ^{32}P radioactivity was released from either the α - or β -subunit of phosphorylase kinase in the 10-min assay. In these conditions, release of ^{32}P radioactivity was linear with respect to time. Protein phosphatase activity is expressed as percentage phosphate released from the substrate relative to that released from either the α - or β -subunit of ^{32}P -labelled phosphorylase kinase.

derived from the other by proteolysis or post-translational modification, includes specific differences in their amino acid compositions^{9,11}, the amino acid sequences around their phosphorylation sites, their two-dimensional phosphopeptide maps and their immunological properties (refs 17, 18 and H.C.H. and P.G., unpublished results). Moreover, DARPP-32 is a phosphatase inhibitor found only in nervous tissue, where it is concentrated in dopaminergic neurones of the basal ganglia⁵⁻⁷, while inhibitor-1 is a more widespread phosphatase inhibitor present in numerous tissues including liver¹⁹, muscle¹¹, adipose tissue²⁰ and brain (H.C.H. and P.G., unpublished results, and ref. 21).

Dopamine alters the state of phosphorylation of several proteins, including DARPP-32, in intact nerve cells by elevating cyclic AMP levels and thereby activating cyclic AMP-dependent protein kinase^{5,6}. The phosphorylation of DARPP-32 by cyclic AMP-dependent protein kinase following the dopamine-mediated increase in cyclic AMP levels provides a mechanism for the inhibition of protein phosphatase-1 by dopamine and cyclic AMP. Because many of the substrates for protein phosphatase-1 are also phosphorylated by cyclic AMP-dependent protein kinase¹³, the activation of DARPP-32 should inhibit their dephosphorylation and thereby amplify the effects of cyclic AMP²². Protein phosphatase-1 also dephosphorylates several phosphoproteins that are phosphorylated by protein kinases other than cyclic AMP-dependent protein kinase¹³, and activation of DARPP-32 presumably allows dopamine, acting through cyclic AMP, to modulate the phosphorylation state of these other substrates also.

A role for protein phosphatase-2B (calcineurin²³) in the regulation of the state of phosphorylation of DARPP-32 is suggested by the efficacy of this phosphatase in dephosphorylating DARPP-32 (Table 2 and ref. 24), as well as by the high levels of both proteins in basal ganglia^{5,6,25}, where they seem to be localized within the same cells^{7,26}. The dephosphorylation of DARPP-32 by protein phosphatase-2B, a Ca^{2+} /calmodulin-activated enzyme²⁷, provides a possible mechanism by which the Ca^{2+} signal might attenuate the dopamine-induced cyclic AMP signal in these cells. Further studies on the role of phospho-DARPP-32 in the control of protein phosphorylation in dopaminergic neurones should provide additional insight into the molecular mechanisms of the trans-synaptic actions of dopamine.

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- Versteeg, D. H. G., van der Gugten, J., de Jong, W. & Palkovits, M. *Brain Res.* **113**, 563-574 (1976).
- Lindvall, O. & Bjorklund, A. *Handbk Psychopharmac.* **9**, 139-231 (1978).
- Hökfelt, T., Johansson, O., Fuxe, K., Goldstein, M. & Park, D. *Med. Biol.* **54**, 427-453 (1976).
- Hökfelt, T., Johansson, O., Fuxe, K., Goldstein, M. & Park, D. *Med. Biol.* **55**, 21-40 (1977).
- Walaas, S. I., Aswad, D. W. & Greengard, P. *Nature* **301**, 69-71 (1983).
- Walaas, S. I. & Greengard, P. *J. Neurosci.* **4**, 84-98 (1984).
- Ouimet, C. C., Miller, P. E., Hemmings, H. C. Jr, Walaas, S. I. & Greengard, P. *J. Neurosci.* **4**, 111-124 (1984).
- Kebabian, J. W. & Calne, D. B. *Nature* **277**, 93-96 (1979).
- Hemmings, H. C. Jr, Nairn, A. C., Aswad, D. W. & Greengard, P. *J. Neurosci.* **4**, 99-110 (1984).
- Huang, F. L. & Glinsmann, W. H. *Eur. J. Biochem.* **70**, 419-426 (1976).
- Nimmo, G. A. & Cohen, P. *Eur. J. Biochem.* **87**, 341-351 (1978).
- Cohen, P. *Nature* **296**, 613-620 (1982).
- Ingebritsen, T. S. & Cohen, P. *Eur. J. Biochem.* **132**, 255-261 (1983).
- Ingebritsen, T. S. & Cohen, P. *Science* **221**, 331-338 (1983).
- Foulkes, J. G., Strada, S. J., Henderson, P. J. F. & Cohen, P. *Eur. J. Biochem.* **132**, 309-313 (1983).
- Stewart, A. A., Hemmings, B. A., Cohen, P., Goris, J. & Merlevede, W. *Eur. J. Biochem.* **115**, 197-205 (1981).
- Hemmings, H. C., Nairn, A. C. & Greengard, P. *J. Biol. Chem.* (submitted).
- Hemmings, H. C., Williams, K. R., Konigsberg, W. H. & Greengard, P. *J. Biol. Chem.* (submitted).
- Goris, J., Defreyn, G., Vandenheede, J. R. & Merlevede, W. *Eur. J. Biochem.* **91**, 457-464 (1978).
- Nemenoff, R. A., Blackshear, P. J. & Avruch, J. *J. Biol. Chem.* **258**, 9437-9443 (1983).
- Detre, J. A., Nairn, A. C., Aswad, D. W. & Greengard, P. *J. Neurosci.* (in the press).

22. Nestler, E. J. & Greengard, P. *Protein Phosphorylation in the Nervous System* (Wiley, New York, 1984).
23. Stewart, A. A., Ingebritsen, T. S., Manalan, A., Klee, C. B. & Cohen, P. *FEBS Lett.* **137**, 80–84 (1982).
24. King, M. M. *et al.* *J. biol. Chem.* (in the press).
25. Wallace, R. W., Tallant, E. A. & Cheung, W. Y. *Biochemistry* **19**, 1831–1837 (1980).
26. Wood, J. G., Wallace, R. W., Whitaker, J. N. & Cheung, W. Y. *J. Cell Biol.* **84**, 66–76 (1980).
27. Stewart, A. A., Ingebritsen, T. S. & Cohen, P. *Eur. J. Biochem.* **132**, 289–295 (1983).
28. Tung, H. Y. L., Resink, T. J., Hemmings, B. A., Shendikar, S. & Cohen, P. *Eur. J. Biochem.* **133**, 455–461 (1983).
29. Tung, H. Y. L., Resink, T. J., Hemmings, B. A., Shendikar, S. & Cohen, P. *Eur. J. Biochem.* **138**, 635–641 (1984).
30. Foulkes, J. G. & Cohen, P. *Eur. J. Biochem.* **105**, 195–203 (1980).
31. Ingebritsen, T. S., Foulkes, J. G. & Cohen, P. *Eur. J. Biochem.* **132**, 263–275 (1983).
32. Peterson, G. L. *Analyt. Biochem.* **83**, 346–356 (1977).
33. Aitken, A., Bilham, T. & Cohen, P. *Eur. J. Biochem.* **126**, 235–246 (1982).

Isolation of HTLV-transformed B-lymphocyte clone from a patient with HTLV-associated adult T-cell leukaemia

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The human T-cell leukaemia/lymphoma virus (HTLV)¹ is an exogenous retrovirus which has been associated with adult T-cell leukaemia/lymphoma (ATL)². This malignancy of T lymphocytes is endemic to southern Japan³, the West Indies⁴, and to a lesser extent, the Middle East, Central Africa and the southeastern United States⁵. ATL cells from patients of diverse geographical origins have been found to be infected with HTLV-1 (ref. 6). HTLV is normally tropic for mature T lymphocytes¹, especially those expressing the helper-inducer surface antigen phenotype⁷ (OKT4 or Leu-3-positive), and the neoplastic T cells infected with HTLV generally express receptors for T-cell growth factor⁸ (detected by reactivity with anti-Tac antibody⁹). However, we report here the isolation of a HTLV-infected B-lymphocyte clone from the peripheral blood of a patient with ATL. This clone is cytogenetically normal and is not infected with Epstein-Barr virus (EBV). Co-culture of cells from this clone with cord blood lymphocytes resulted in transmission of HTLV and the immortalization of either T or B lymphocytes. These results suggest that HTLV may be associated with a broader range of host cells than previously recognized.

We were attempting to culture the malignant T cells from a patient with HTLV-associated ATL. The patient had refractory hypercalcaemia, diffuse lymphadenopathy, bilateral interstitial pulmonary infiltrates, and a peripheral white blood cell count of 63,500 cells mm⁻³, 98% of which were malignant, T4-positive, Tac antigen (T-cell growth factor receptor)-positive T lymphocytes that bore the typical morphological features of adult T-cell leukaemia/lymphoma⁵.

The patient's peripheral blood mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation and cultured at 5 × 10⁶ cells per ml of ER medium (comprising 43 ml Eagle's high amino acids medium, 43 ml RPMI 1640, 10 ml heat-decomplemented fetal calf serum, 2 ml 200 mM L-glutamine, 1 ml 10 mg ml⁻¹ gentamicin, 1 ml 1 mg ml⁻¹ penicillin, and 0.05 ml 70 µM 2-mercaptoethanol in 100 ml) without other added growth factors. After 3 weeks in culture at 37 °C in 5% CO₂, a cell line grew spontaneously that was then cloned by limiting dilution at 0.3 cells per well. Both the initial cell line (HS) and a representative clone (HS-1) expressed monoclonal IgM λ antibody. Table 1 shows a profile of the surface characteristics of HS-1. HS-1 is a B lymphocyte expressing immunoglobulin, Ia molecules and the B-cell differentiation markers B1 (ref. 10), BA1 (ref. 11), and BA2 (ref. 11) but it is

negative for the common acute lymphoblastic leukaemia antigen. It does not express the T-cell markers that were noted on the patient's malignant T cells (T4, 3A1 or T101). HS-1 expresses the HTLV-encoded viral protein p19 (ref. 12) on its surface and cell extracts contain viral reverse transcriptase¹³. HS-1 is not infected with EBV as demonstrated by the absence of EBV nuclear antigen. Unlike normal B lymphocytes, HS-1 expresses the receptor for T-cell growth factor (TCGF) on its surface. Preliminary characterization of this receptor suggests that it is identical in size to that normally expressed on activated T cells (S. Korsmeyer *et al.*, manuscript in preparation) but despite its expression the cells do not require TCGF to proliferate. However, the addition of various concentrations (1%, 5% and 10%) of human TCGF (Cellular Products) to the culture medium results in a modest augmentation of proliferation at the highest concentration of TCGF tested (24% increase in ³H-thymidine incorporation). Furthermore, HS-1 does not secrete TCGF into the culture medium as HS-1 conditioned medium fails to support the growth of the TCGF-dependent mouse T-cell line, HT-2. Cytogenetic analysis revealed that HS-1 has a normal female karyotype with the patient's malignant T-cells bearing abnormalities in 72% of the metaphases, the predominate clonal karyotype (44% of the abnormal cells) being 46, XX, t(1;6) (p31;q11), reciprocal(8;12) (q24;q14).

To demonstrate further the HTLV transformation of HS-1 cells, we incubated irradiated (12,000 R) HS-1 cells with cord blood lymphocytes in three different conditions: (1) simple co-culture of irradiated HS-1 and cord blood lymphocytes, (2) co-culture with the addition of 4 µg ml⁻¹ concanavalin A (Con A) to the medium, and (3) addition of irradiated HS-1 after 3 days of Con A stimulation of the cord blood lymphocytes. The transformed cell lines obtained from each of the culture conditions were termed CS-1, CS-2 and CS-3, respectively. The cell surface characteristics of these cell lines are listed in Table 1. CS-1 cells appear to be Leu-3⁺, Tac⁺, Ia⁺ T lymphocytes that express the viral p19 protein. CS-2 cells express IgM κ surface immunoglobulin, B1, Tac, Ia and viral p19. CS-3 cells have features of both T and B cells and express Leu-3 as well as IgM λ surface immunoglobulin, Tac, Ia and viral p19. The isoenzyme phenotype of HS-1, CS-1, CS-2 and CS-3 demonstrates that HS-1 and the cord blood lymphocyte lines originated from two different people (data not shown). The co-cultured cell lines were also Epstein-Barr nuclear antigen (EBNA)-negative.

In order to characterize the integration sites of the virus in the fresh leukaemic T cells, the HS-1 B-cell clone and the co-cultured cord blood lymphocyte cell lines, and to substantiate further the identity of the virus, we performed Southern analysis on DNA isolated from these cells¹⁴. Virus integrations can be analysed by digesting the cell DNA with EcoRI which does not

Table 1 Cell surface phenotype

		Parent HS-1	Co-culture cell lines		
			CS-1	CS-2	CS-3
Leu-3		—	+	—	+
p19	40%	+	+	+	+
Tac	60%	+	+	+	+
B1	84%	+	—	+	—
3A1		—	+	—	—
IgG		—	—	—	—
IgA		—	—	—	—
IgM	65%	+	—	+	+
IgD		—	—	—	—
κ		—	—	+	—
λ	77%	+	—	—	+
DR	95%	+	+	+	+
cALLa		—			
BA1	57%	+			
BA2	21%	+			

Summary of cell surface phenotype determined by fluorescence-activated cell sorter. + Denotes 40% or more of cells reacting with the antibody; — denotes 5% or less of cells reacting with the antibody.

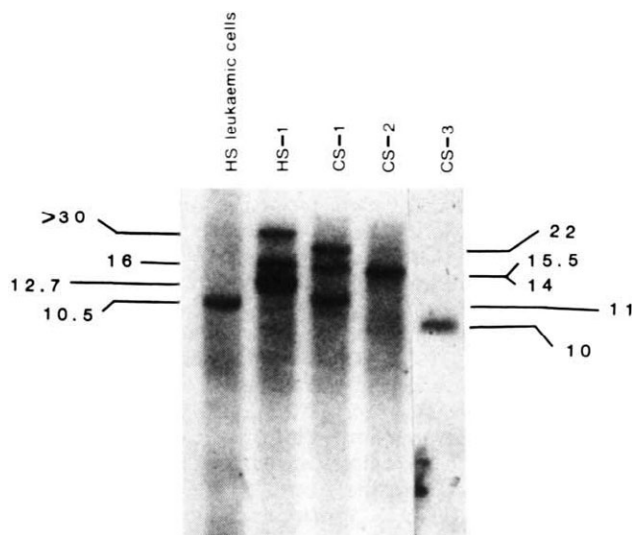


Fig. 1 Characterization of HTLV-I integration sites in HS and transmission cell lines. DNA (10 µg) was cleaved with *Eco*RI and electrophoresed on a 0.8% agarose gel. The gels were blotted to nitrocellulose and hybridized. The probe used was pHTLV₂₃, a pUC8 subclone of the HTLV genomic clone previously described⁶. Proviral bands are indicated and sizes in kilobases are shown.

cleave HTLV-I proviral DNA⁶, thus showing provirus integrations at different sites in the genome. The viral integration sites in the uncloned HS line and the malignant T-cell clone are different (Fig. 1). Analysis of viral integrations in the cloned HS-1 line and the uncloned transformed cord blood lines showed that in each case the integration site differed and thus represented a unique infection with HTLV (Fig. 1). HS-1 had three integrated proviral copies; CS-1 had three; CS-2 and CS-3 each had one. We also performed restriction analysis with *Bam*HI and *Pst*I which showed the expected internal cleavage fragments generated from an HTLV-I genome (data not shown)¹⁵.

The expression of the TCGF receptor on HS-1 and the absence of this receptor on any normal resting B lymphocytes suggest that TCGF receptor gene activation and receptor protein synthesis may be a consequence of HTLV infection. On the other hand, preliminary data from our laboratory and elsewhere show that activated normal B cells may express the TCGF receptor after stimulation with a variety of B-cell mitogens (A. Muraguchi *et al.*, manuscript in preparation). It is possible that the TCGF receptor is the site of virus binding by B lymphocytes, as has been suggested for T cells¹⁶.

It has been reported previously that B cells from patients with HTLV-associated T-cell malignancies do not contain HTLV-related DNA sequences^{17,18}, but the control B cells for these experiments were EBV-transformed cells from ATL patients. However, given that a single B-cell clone emerged *in vitro* in our experiments, it is conceivable that the precursor frequency of HTLV-susceptible B cells is substantially lower than that susceptible to EBV transformation. Yamamoto *et al.*¹⁸ have isolated a number of EBV-transformed B-cell lines from Japanese patients with HTLV-associated ATL and a small fraction of these B cells appear to express HTLV viral proteins. However, no transmission data were presented.

It seems that B-cell transformation by HTLV may be more common than previously suspected. Independently, D. Mann *et al.* (personal communication) have found evidence for B-cell transformation by HTLV in another patient with HTLV-associated ATL although they have not yet completely characterized the transmissibility of the HTLV.

Thus, we have isolated a cytogenetically normal, EBV-negative, B-lymphocyte clone that has been transformed by infection with HTLV. We suggest that the B-cell clone became infected

with HTLV *in vitro* from the patient's malignant T cells. It is not clear whether this particular strain of HTLV-I (HTLV-I_{HS}) has unique structural features that permit a broader host range than previous isolates or whether the particular B cell that became infected has features (such as virus-binding immunoglobulin or TCGF receptor expression) that facilitated the transformation by HTLV-I. Studies are currently underway to evaluate these possibilities²⁰.

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1. Poiesz, B. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 7415-7419 (1980).
2. Blayney, D. W. *et al. Ann. intern. Med.* **98**, 144-147 (1983).
3. Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K. & Uchino, H. *Blood* **50**, 481-492 (1977).
4. Catovsky, D. *et al. Lancet* **i**, 639-643 (1982).
5. Blayney, D. W. *et al. Blood* **62**, 401-405 (1983).
6. Wong-Staal, F. *et al. Nature* **302**, 626-628 (1983).
7. Popovic, M. *et al. Science* **219**, 856-859 (1983).
8. Gallo, R. C. *et al. UCLA Symp. molec. cell. Biol. New Ser.* **1**, 231-246 (1982).
9. Uchiyama, T., Broder, S. & Waldmann, T. A. *J. Immun.* **126**, 1393-1397 (1981).
10. Stashenko, P., Nadler, L. M., Hardy, R. & Schlossman, S. F. *J. Immun.* **125**, 1678-1685 (1980).
11. Abramson, C. S., Kersey, J. S. & LeBien, T. W. *J. Immun.* **126**, 83-88 (1981).
12. Robert-Guroff, M., Ruscetti, F. W., Posner, L. E., Poiesz, B. J. & Gallo, R. C. *J. exp. Med.* **154**, 1957-1964 (1981).
13. Rho, H. M., Poiesz, B. J., Ruscetti, F. W. & Gallo, R. C. *Virology* **112**, 355-360 (1981).
14. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
15. Clarke, M. F., Gelmann, E. P. & Reitz, M. S. *Nature* **305**, 60-62 (1983).
16. Lando, Z. *et al. Nature* **305**, 733-736 (1983).
17. Gallo, R. C. *et al. Proc. natn. Acad. Sci., U.S.A.* **79**, 5680-5683 (1982).
18. Yamamoto, N., Matsumoto, T., Koyanagi, Y., Janaka, Y. & Hinuma, Y. *Nature* **299**, 367-369 (1982).

Presence of T-cell receptor mRNA in functionally distinct T cells and elevation during intrathymic differentiation

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Understanding the differentiation of functionally distinct subsets of T lymphocytes is essential to unravel their crucial role in the immune response and awaits knowledge of the assembly and expression of genes encoding the T-cell receptor. Recently, we have cloned and sequenced complementary DNA that may specify part of the human T-cell receptor¹. The deduced protein sequence showed extensive similarity to the entire length of mammalian immunoglobulin light chains¹. In addition, sequences corresponding to this message undergo somatic rearrangements² and are assembled from non-contiguous genomic sequences into a single mRNA molecule³, a mechanism similar to those found in the generation of immunoglobulin messages⁴. A related molecule from the mouse was also isolated independently by Hedrick *et al.*⁵. Here we show that the putative T-cell receptor mRNA is expressed at a relatively high level during intrathymic differentiation before decreasing about 10-20-fold in normal, mature peripheral blood T cells and that it can also be detected in T-cell clones with helper and cytotoxic functions, as well as in at least one clone with suppressor properties.

We have assessed the RNA level by Northern gel analysis⁶. RNA was extracted from human leukaemic T-cell lines, human thymocytes⁷, peripheral blood T cells⁷, phytohaemagglutinin (PHA) stimulated T cells⁷, bone marrow cells⁷ and Epstein-Barr

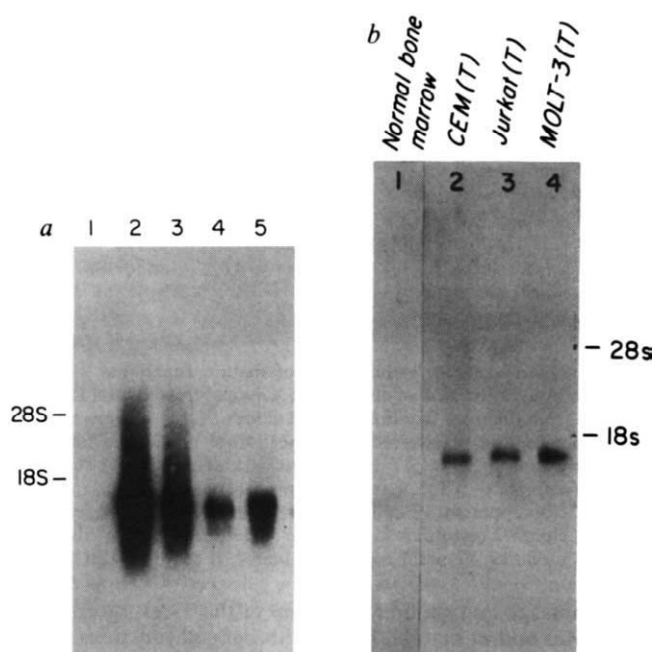


Fig. 1 Northern blot analysis of immature and mature T-cell RNA homologous to sequences of the putative T-cell receptor clone YT35. RNAs were extracted from different T cells in the presence of guanidine thiocyanate, and treated with glyoxal¹. 8 µg each of total RNA was electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, pH 7.0. Transfer of the RNA to nitrocellulose membranes was carried out as described previously⁶. Hybridization was to ³²P-dCTP-labelled, nick-translated 0.9 kb *Nco*I-*Nco*I fragment of YT35⁶ which is internal to the cDNA insert. *a*, RNA is from: lane 1, a human B-cell line, HSC-58; lane 2, a human T-cell line, MOLT-3; lane 3, total human thymocytes (provided by Dr E. Gelfand); lane 4, PHA-stimulated human T lymphocytes⁷; lane 5, E⁺ rosette peripheral lymphocytes⁷. *b*: lane 1, total normal bone marrow cells; lane 2, leukaemia cell lines CEM; lane 3, Jurkat; lane 4, MOLT.

virus-transformed lymphoblastoid B-cell lines (LBCL)¹. Results in Fig. 1 show the presence of a major molecule of about 1.3 kilobases (kb) in all types of T cells (a minor molecule at 1.1 kb can also be observed in some cases). The level of RNA is highest in leukaemic T-cell lines, CEM (Fig. 1*b*, lane 2), Jurkat (Fig. 1*b*, lane 3), MOLT-3 (Fig. 1*a*, lane 2; Fig. 1*b*, lane 4) and thymocytes (Fig. 1*a*, lane 3). A lower level of RNA, however, is present in the more mature peripheral blood T cells, whether resting (Fig. 1*a*, lane 5) or phytohaemagglutinin-stimulated (Fig. 1*a*, lane 4), while no RNA homologous to YT35 was found in control B-cell lines (Fig. 1*a*, lane 1) or bone marrow cells (Fig. 1*b*, lane 1). These results confirm the identification of the YT35 message as a T-cell-specific gene¹.

To quantitate the relative level of the RNA in different types of T cells, dot hybridization analysis was also performed. The data in Fig. 2*a* and *b* demonstrate further that the difference in the level of mRNA between the relatively more immature T cells (leukaemic T-cell lines and thymocytes) and mature T cells is about 10-fold. These differences are found for both the complete YT35 mRNA¹ and the mRNA corresponding to the 'constant' region probe¹.

The elevated level of mRNA for the putative T-cell receptor in the thymus may characterize certain ontogenetic phases of T-cell differentiation or it may be specific for thymocytes. In an attempt to distinguish between these two possibilities, we have induced leukaemic T-cell lines (MOLT-3 and Jurkat), lacking mature T-cell markers, to differentiate and express such markers using the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)^{7,8}. Northern-gel and dot-blot analysis of the level of the YT35 mRNA showed that the band was of about the same size and intensity in the induced and in the uninduced

Table 1 Characterization of alloreactive T-cell clones

Clone	Phenotype subset		Blastogenic response to		Effect on B cell	
	OKT4	OKT8	DR1 ⁺ Stimulators	DR1 ⁻ Stimulators	CML activity	Responses to SRBC
-19	+	-	+	-	None	Helper
-22	+	-	+	-	None	Helper
-207	+	-	+	-	Anti-DR1	Helper
-209	+	-	+	-	None	Suppressor
-26	-	+	-	-	Anti-B35	None

Alloreactive T-cell clones were generated by limiting dilutions of MLC blasts obtained from a HLA-A2, A33, B7, B14, DR3, DR7 responder primed to a HLA-A2, A11, B8, B35, DR1, DR3 stimulator. The clones were maintained in interleukin-2-containing culture medium by weekly stimulation of the clones with irradiated, Epstein-Barr virus-transformed lymphoblastoid B-cell lines (LBCL) of immunized origin¹⁷. The phenotype of the clones was assessed on a Spectrum III cytofluorometer (Ortho) using fluoresceinated murine monoclonal OKT4 and OKT8 antibodies (Ortho)¹⁸. The clones were tested for anti-HLA-D/DR MLC reactivity to HLA-D homozygous typing cells in a 72-h blastogenesis ³H-thymidine incorporation assay. Cytolytic T-cell assays were performed by the ⁵¹Cr-release method¹⁹. Allogeneic helper function was assayed as a primary response to sheep erythrocytes (SRBC)^{20,21}.

MOLT-3 and Jurkat cells. This indicates that induction of differentiation by TPA is not accompanied by any significant change in the abundance of YT35 mRNA.

Although it has been shown that different regulatory and effector functions can be carried out by distinct subsets of T cells characterized by unique cell-surface antigens, a complicated network of interactions exists within and between each subset^{9,10}. With the advent of cell cloning procedures, T-cell lines with specialized functions and distinct phenotypes have been established and characterized. We have therefore assessed the level of the YT35 mRNA in alloreactive T-cell clones with different, well-characterized functions¹¹ (Table 1). Results in Fig. 3 indicate that RNA extracted from all the five T-cell clones contain messages homologous to the cDNA YT35 of about 1.3 and 1.1 kb in size. These results illustrate that RNA corresponding to the putative T-cell receptor is expressed in all three classes, that is helper, cytotoxic and suppressor (at least one clone). The level of RNA expression may vary in different T-cell clones (Table 1). The highest amount is found in clone 209 (suppressor; lane 2), followed by clone 26 (cytotoxic; lane 5), clone 19 (helper; lane 6) and clone 207 (cytotoxic-helper; lane 3), while the lowest amount was found in clone 22 (helper; lane 4). No hybridization was observed from the B-cell line derived from the same individual (lane 7). It is not clear, however, whether these differences in the level of expression in the T-cell clones are related to the functional characteristics of the clones or whether they represent fluctuations determined by other factors. As the doubling time of the five different clones was about the same (24–36 h), it is unlikely that the rate of clonal expansion influenced the level of expression. Furthermore, as these are twin clones derived from the same mixed lymphocyte culture, individual (responder-specific) variations do not seem to be involved.

The results summarized here demonstrate that the mRNA corresponding to the putative T-cell receptor clone YT35 is present in all types of T cells (thymus, peripheral blood and T-leukaemia) including T-cell clones with specific immune functions. This message is absent from normal bone marrow, B cells and other haematopoietic or non-haematopoietic cells¹. The finding that all T-cell lines examined expressed this message suggests that if the T-cell receptor is, as proposed, a heterodimer of two chains^{12–14}, YT35-encoded protein is an integral part of it. The level of the YT35 messages in immature leukaemic T lymphoblasts and thymocytes is about 10-fold higher than the level found in mature T lymphocytes. It is unlikely that such differences merely reflect incomplete hybridization due to nucleotide mismatches in the 'variable' regions of different cell clones. If this were the case, there would be no difference between the level of mRNA observed with the complete YT35 (ref. 1) and when only the 'constant' region¹ of the YT35 probe

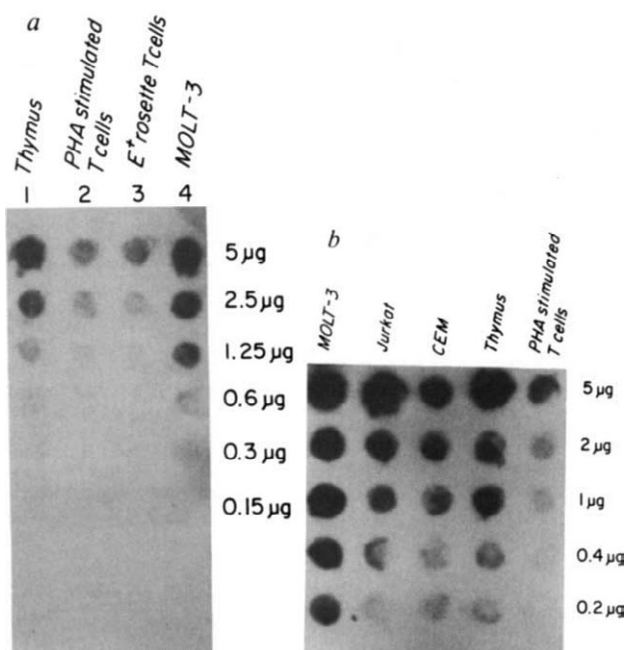


Fig. 2 Dot blot analysis⁶ of DNA from immature and mature T cells. Different amounts of total RNA (5, 2.5, 1.25, 0.6, 0.3 and 0.15 µg) from various types of T cells were spotted onto nitrocellulose paper. This was hybridized with ³²P-dCTP-labelled, nick-translated YT35 insert⁶ (0.9 kb *Nco*I-*Nco*I fragment). *a*, Lane 1, human thymocytes; lane 2, PHA-stimulated human T lymphocytes; lane 3, E-rosette positive T cells; and lane 4, MOLT-3 cells. *b*, Lane 1, MOLT-3; lane 2, Jurkat; lane 3, CEM; lane 4, thymocytes; and lane 5, PHA-stimulated human T cells.

was used. It is also unlikely that the differences reflect incomplete hybridization due to nucleotide mismatches in the 'constant' regions of different cell clones. If this were so, one would not expect thymocytes to have an elevated level as they would be likely to be heterogeneous with respect to the T-cell receptor chain. Furthermore, all immature T-cell leukaemic lines expressed higher levels of RNA than did the mature T-cell clones examined, thus indicating that the latter exhibit very similar if not identical 'constant' regions. Preliminary results support the view that the nucleotide sequences of the constant region of the different T-cell lines are very similar (Y.Y., unpublished data).

Thus, in sharp contrast to the genetic information encoded in immunoglobulin genes, which are expressed at significantly higher levels in plasma cells than in pre-B cell lines¹⁵, the T-cell receptor message is more abundant during intrathymic differentiation than in post-thymic T-cell lineages.

These observations raise some interesting speculations on the function(s) and possible role of the protein in immature T cells. The finding that these sequences are expressed not only at significantly higher levels in the relatively more immature T cells but that they seem to remain elevated throughout intrathymic differentiation suggests that the T-cell receptor and its RNA have an important role in early stages of T-cell ontogeny. If, as proposed¹⁶, thymic T cells express receptors for self, it is conceivable that high levels of expression will permit a more efficient elimination of autoreactive T-cell clones.

Another major finding in our study is the observation that all the T-cell clones—with the phenotype and properties of help, cytotoxicity and, in at least one clone, suppression (as defined by the biological assays indicated¹¹)—express this message. This finding suggests that helper and cytotoxic T cells and at least some suppressor cells operate via receptor molecules which mediate either regulatory functions (such as the activation or suppression of the cellular and humoral immune responses) or effector-cytolytic functions. The generation of diversity within the T-cell population might be elucidated through the analysis

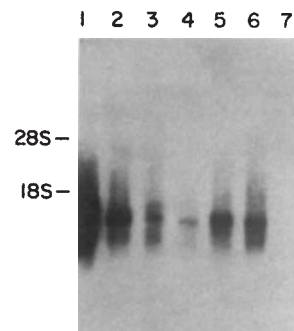


Fig. 3 Northern blot examination of mature functional T-cell clones for hybridization of clone YT35. Glyoxal-treated total RNA (8 µg) from different functional T-cell clones (Table 1) were electrophoresed and transferred to nitrocellulose membrane filters. Hybridization to ³²P-dCTP-labelled nick-translated 0.9 kb, *Nco*I-*Nco*I fragment of YT35. Lane 1, peripheral blood T cells; lane 2, clone 209 (suppressor); lane 3, clone 207 (helper T cells); lane 4, clone 22 (cytotoxic killer); lane 5, clone 26 (cytotoxic killer); lane 6, clone 19 (helper T cells); and lane 7, B cell line (LBCL8).

of the message and genomic structure of the T-cell receptor in thymocytes and in mature T cells with defined functions.

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1. Yanagi, *et al.* *Nature* **308**, 145-149 (1984).
2. Toyonaga, B. *et al.* *Nature* (in the press).
3. Siu, J. *et al.* *Cell* **37**, 393-401 (1981).
4. Honjo, T. *A. Rev. Immun.* **1**, 499-528 (1983).
5. Hendrick, S. M., Nielsen, S. M., Kavalier, J., Cohen, D. T. & Davis, M. *Nature* **308**, 153-158 (1984).
6. Thomas, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
7. Nagasawa, K. *et al.* *Thymus* **3**, 307-312 (1982).
8. Nagasawa, K. & Mak, T. W. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2964-2968 (1980).
9. Ballieux, R. E. & Jeijnen, C. J. *Immun. Rev.* **74**, 29-56 (1983).
10. Steinmetz, M. & Hood, L. *Science* **222**, 727-733 (1983).
11. Rohowsky, C., Lewison, A., Bonagura, V., King, D. W. & Suciu-Foca, N. *Hum. Immun.* (in the press).
12. Acuto, O. *et al.* *Cell* **34**, 717-726 (1983).
13. Kappler, J. *et al.* *Cell* **34**, 727-737 (1983).
14. McIntyre, B. W. & Allison, J. P. *Cell* **34**, 739-746 (1983).
15. Herzenberg, L. A., Hayakawa, K., Hardy, R. A., Tokuhisa, T. & Oi, V. T. *Immun. Rev.* **67**, 5-32.
16. Jerne, N. K. *Eur. J. Immun.* **1**, 1-9 (1971).
17. Suciu-Foca, N., Rohowsky, C., Kung, P. & King, D. W. *Hum. Immun.* **9**, 37-47 (1984).
18. Suciu-Foca, N., Rohowsky, C., Kung, P. & King, D. W. *J. exp. Med.* **156**, 283-288 (1982).
19. Krensky, A. M., Reiss, C. S., Mier, J. W., Strominger, J. L. & Burakoff, S. J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2365-2369 (1982).
20. Fauci, A. C. & Pratt, K. R. *J. exp. Med.* **144**, 674-683 (1976).
21. Fauci, A. C. & Pratt, K. R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3676-3679 (1976).

Transformation of NIH 3T3 cells by microinjection of Ha-ras p21 protein

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Alteration in gene structure has been shown to occur in some human tumours¹⁻⁵. These altered genes, termed oncogenes, were originally identified by their ability to induce foci of transformed cells on transfected mouse 3T3 cultures. The oncogene identified in the EJ/T24 human bladder carcinoma is similar to the transforming gene of BALB and Harvey murine sarcoma virus (MSV)^{6,7} and differs from its counterpart in normal cells by a single amino acid⁸. All three of these Ha-ras genes direct the production of

similar proteins (p21). While the *ras* gene appears to be involved in tumour formation in some situations, its role is unclear. The *ras* protein product (p21) binds guanine nucleotides and has a unique autophosphorylating activity⁹⁻¹², but no other enzymatic activity has been found. We report here the injection of purified Ha-*ras* p21 protein¹³⁻¹⁶, made in *Escherichia coli* from the gene of BALB-MSV, into NIH 3T3 cells and show that the purified protein itself is sufficient to induce a transformed morphology. In addition, the injected protein stimulates quiescent cells to enter the S-phase of the cell cycle. This result clearly demonstrates that the *ras* gene functions directly through the protein product. It also establishes an assay for the protein which depends on its activity within a living cell. The transforming activity of a p21 *ras* protein equivalent to the product of the normal cellular *ras* gene, is also demonstrated.

The BALB-MSV p21 *ras* protein has recently been produced in *E. coli*¹⁶. The protein was made with high efficiency and could be purified to near homogeneity by a differential membrane extraction procedure¹⁶. It is highly hydrophobic, presumably due to its membrane localization, and soluble up to $\sim 3 \text{ mg ml}^{-1}$, but only in the presence of 3 M guanidine-HCl. A guanidine solution, however, is unlikely to be tolerated in injected cells. It is desirable to inject a concentrated protein preparation as the injected solution is diluted ~ 20 -fold on entry into the cytoplasm¹⁷. In this study, proteins were solubilized at high concentration by addition of a 10-fold excess of bovine serum albumin (BSA) to the *ras* protein in the absence of guanidine-HCl (see Fig. 1).

The BALB-MSV *ras* protein was microinjected into many cells in an area designated by marks on the back of the coverslip. After 24 h, cells in the injected area had clearly undergone a morphological transformation; they became rounded and refractile. Injection of BSA alone, however, had no effect on cell morphology (Fig. 1). To ensure that injections and subsequent analysis were reproducible, all injections described below were performed with coded samples.

A time course analysis was undertaken to elucidate the morphological alterations induced by the *ras* protein. Cells were injected and observed for 4 days. At 20 min after injection, recipient cells appeared entirely normal. Morphological changes were not discernible until 12 h after injection, but by 16 h had become quite distinct (Fig. 1). The transformed morphology was apparent for at least 24 h and the cells then gradually reverted to the flattened appearance of uninjected cells. In some cases, transformation was maintained for up to 40 h after injection, but was never observed after 2 days. Occasionally, cells in the area previously injected appeared more densely populated than normal after reversion to the normal morphology, suggesting that increased cell division had occurred. This observation was substantiated by the appearance of two- to over sevenfold more mitotic cells in the injected area than normal (data not shown). No such alterations resulted following BSA injection.

A dose-response analysis was performed after preparation of a solution of *ras* protein at 20 mg ml^{-1} (with 200 mg ml^{-1} BSA). Dilutions of 3-, 9- and 27-fold were then prepared and injected separately, with BSA as a negative control. All three *ras* protein-containing solutions induced transformation, but the 9-fold dilution (2.2 mg ml^{-1}) gave the most marked results. Injections of this material resulted in the appearance of $\sim 6 \times 10^7$ molecules of *ras* p21 in each cell (Fig. 1). The lowest concentration (0.7 mg ml^{-1}) produced less obvious transformation while the higher concentration (6.7 mg ml^{-1}) produced cytopathic effects and cell transformation (results not shown). The cytopathic effects may be due to the *ras* protein, the higher concentration of BSA or an excess of salt in the solution. As noted previously, no transformation was observed following BSA injections or *ras* protein below 0.2 mg ml^{-1} . Several other cell types have also been tested—including the diploid human cell line MRC5, the human skin line Detroit 550 (obtained from S. D. Makover), and secondary culture chick and mouse embryo cells (data not shown)—but have failed to display distinct morphological transformation following *ras* p21 injection.

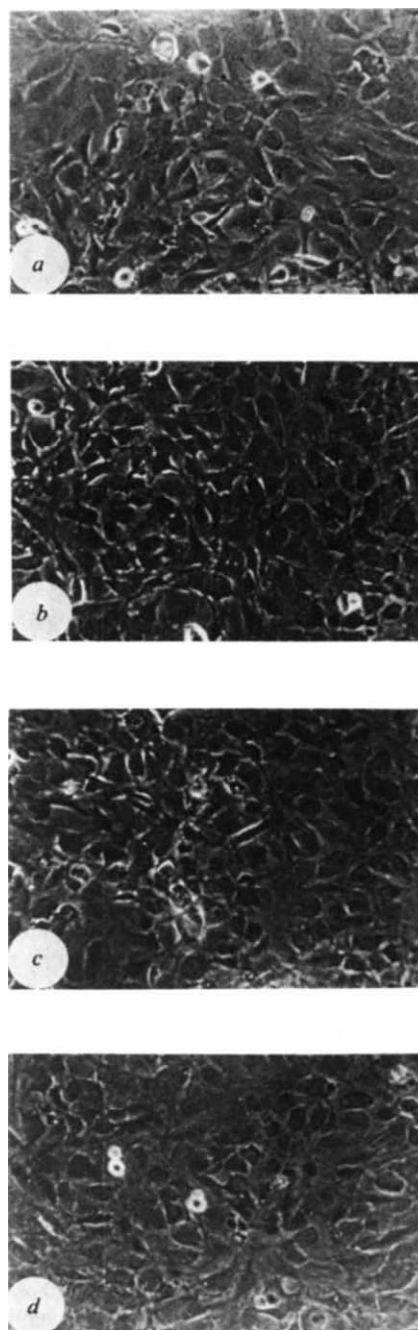


Fig. 1 Morphological appearance of cells injected with BALB *ras* p21 protein or its normal counterpart. The BALB *ras* p21 at 0.7 mg ml^{-1} (c) or 2.2 mg ml^{-1} (b) was injected into cells within the left half of the area photographed. Uninjected cells are at the right. The *ras* p21 protein which represents the normal human counterpart of the transforming *ras* gene was injected at 3.3 mg ml^{-1} (a), while BSA (22 mg ml^{-1}) was injected as negative control (d). The BALB *ras* protein was prepared from an *E. coli* clone with plasmid pJCL-E30 which contains the BALB-MSV transforming gene under the control of the bacteriophage P_L promoter. Protein was prepared by successive membrane extractions, as described previously¹⁶, and represented one major band on SDS-gel electrophoresis¹⁶. The purified *ras* protein was mixed with a 10-fold excess of BSA (crystallized; Sigma) and prepared for injection as described in the text. The normal *ras* protein was prepared from an *E. coli* clone containing plasmid pJCL-33¹⁶. This plasmid was derived by substituting 55 base pairs of the normal rat gene (from *Hind*III to *Pvu*II sites) into the plasmid containing the viral gene. In this way the position known to be altered in the transforming *ras* gene is reverted to the normal sequence. Microinjection procedures are described elsewhere²².

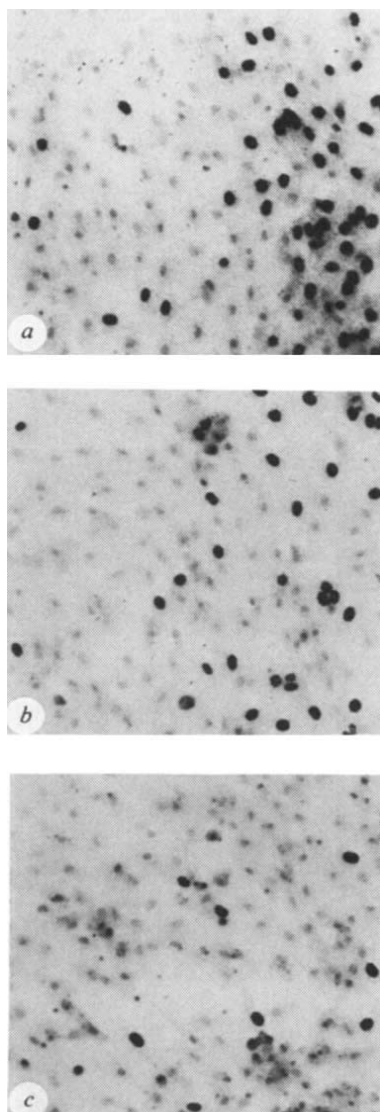


Fig. 2 Thymidine incorporation following injection of *ras* p21. *a* Ha-*ras* (2.2 mg ml^{-1}) was injected into numerous cells at the right of the area photographed. The appearance of cells to the left indicates the behaviour of uninjected cells. *b*, the normal *ras* protein (3.3 mg ml^{-1}) or bacterial protein preparation (3.3 mg ml^{-1}) (*c*) were also injected into cells at the right of the areas photographed. Injected cells were allowed to grow to confluence before culture in medium containing 2% serum for 6 days before injection. From 16 to 24 h after injection, $10 \mu\text{Ci } ^3\text{H}$ -thymidine was added to the culture which was then fixed with 2% glutaraldehyde and autoradiographed with nuclear tracking emulsion (Kodak). The cells were then lightly stained with haematoxylin. The dark nuclei identify those cells in which ^3H -thymidine had been incorporated into DNA.

We next studied the activity of the normal human counterpart to the Ha-*ras* gene. It has been shown that a high level of expression of the normal human *ras* gene can also induce transformation. When NIH 3T3 cells were transfected with this cloned gene linked to a highly active viral long terminal repeat promoter, transformation was observed, but with less efficiency than observed with Harvey sarcoma viral DNA¹⁸. The non-mutated Ha-*ras* gene has also been expressed in *E. coli* and the protein purified as described previously¹⁶. To confirm that high levels of the non-mutated protein are sufficient to induce transformation, this protein was injected into 3T3 cells as described above. In each of several separate experiments, transformation of injected cells was observed, but only with protein at

Table 1 Thymidine incorporation following Ha-*ras* p21 injection

Sample	Expt	No. of cells	% Positive
Ha- <i>ras</i> p21	1	97	29
	2	110	54
	3	144	57
Control	1	105	4
	2	156	1
	3	98	5

The percentage positive cells was determined by subtracting the background percentage of uninjected cells on the coverslip which incorporated thymidine.

3.3 mg ml^{-1} . Less concentrated solutions were unable to induce the transformed phenotype. Even with the concentrated solution, transformation was not as pronounced as with the transforming BALB-MSV *ras* protein (Fig. 1*a*). This result indicates that both the normal and altered *ras* protein produce similar effects but that the altered protein is effective at a lower concentration.

The protein preparations injected in these studies were produced in bacteria and would therefore not be contaminated by eukaryotic proteins (other than those present in the crystalline carrier BSA). Contaminating prokaryotic proteins, however, might have been responsible for the results seen, even though the *ras* protein preparations ($>80\%$ pure) were shown to be free of any major contaminants. To ensure that contamination was not responsible for the results described, three unrelated eukaryotic proteins, of similar size, expressed in bacteria and purified in exactly the same way (S. Yamazaki and H. F. K., in preparation) as *ras* protein¹⁶, were injected into NIH 3T3 cells; there was no evidence of transformation following any of these injections (results not shown). Ha-*ras* p21 is cleaved at position 33 by treatment with formic acid. The cleaved protein was also unable to transform NIH 3T3 cells (results not shown). We therefore conclude, that the *ras* protein present in injected preparations was responsible for the biological result observed.

The influence of BSA co-injected with the *ras* protein preparations was also considered. Conjugated and unconjugated serum albumins have been carefully studied following injection into HeLa cells and rat fibroblasts. In both cell types the injected BSA was segregated into localized areas within the cytoplasm, entered lysosomes within 10 h and gradually disappeared from the cell over the next 24 h¹⁹. It seems likely, therefore, that following injection, the BSA is degraded and the *ras* protein associates with cellular proteins. The fact that the transformed phenotype remains for nearly 2 days suggests that the *ras* protein itself is not rapidly degraded by the cell. This is not surprising, as injected proteins can remain within injected cells for several days¹⁹. Alternatively, the injected protein might trigger cellular changes which persist after the protein has disappeared. Studies of *ras* protein stability are in progress.

Morphological transformation is a readily identifiable parameter and microinjection of p21 protein seems to be a very sensitive assay of protein activity. Alterations in morphology following injection, however, have the disadvantage that microinjection itself may alter morphology, at least transiently, although it does not necessarily harm a cell. For example, translation, transcription and many other properties of injected cells occur faithfully within the first hour following injection²⁰. It would nevertheless be useful to identify another characteristic of transformation exhibited following p21 *ras* protein injections to confirm the morphological observations. As *ras* transformed cells are known to have reduced serum requirements for growth, we investigated whether injection of *ras* protein could induce cells to initiate DNA synthesis in serum-depleted medium.

NIH 3T3 cells were grown in medium containing reduced serum to decrease the rate of cell division to a low level²¹. An

area of the coverslip was marked and numerous cells in that area were injected. ^3H -thymidine was added to the medium 16–24 h after injection and the cells were then fixed and autoradiographed. In the area of cells injected with Ha-ras protein, numerous cells incorporated label into their nuclei. Fewer cells had entered S-phase following the injection of the normal ras protein, while few if any had begun to incorporate ^3H -thymidine due to injection of an unrelated protein purified from bacteria (Fig. 2). The probability that an individual cell would incorporate thymidine was determined by attempting to inject all cells within a defined area of the coverslip. In three experiments, approximately half the ras-injected cells entered S-phase while few cells injected with a control protein preparation responded (Table 1). The thymidine incorporation assay is of value because it tests a second property of transformed

cells, the results are definite and it may be more quantitative than morphological alterations. In uninjected cultures induced to enter S-phase by the addition of serum, thymidine incorporation was similar to that observed with injected cells, indicating that the incorporation observed did not result from DNA repair.

These studies clearly identify the p21 ras protein as directly responsible for morphological and growth-related changes observed in cells transformed by the ras gene. They also establish two distinct assays for the transforming properties of the protein. We hope such assays will facilitate structure-function studies of the ras protein.

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1. Goldfarb, M., Shimizu, K., Peruchio, M. & Wigler, M. *Nature* **296**, 404–409 (1982).
2. Tabin, C. *et al.* *Nature* **300**, 143–149 (1982).
3. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. *Nature* **300**, 149–152 (1982).
4. Yuasa, Y. *et al.* *Nature* **303**, 775–779 (1983).
5. Sukumar, S., Notario, V., Martin-Zanca, D. & Barbacid, M. *Nature* **306**, 658–661 (1983).
6. Bishop, J. M. *A. Rev. Biochem.* **52**, 301–354 (1983).
7. Cooper, G. M. *Science* **217**, 801–806 (1982).
8. Capon, D. J., Chen, E. Y., Levinson, A. D., Seeburg, P. H. & Goeddel, D. V. *Nature* **302**, 33–37 (1983).
9. Scolnick, E. M., Papageorge, A. G. & Shih, T. Y. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5355–5359 (1979).

10. Shih, T. Y., Papageorge, A. G., Stokes, P. E., Weeks, M. D. & Scolnick, E. M. *Nature* **287**, 686–691 (1980).
11. Papageorge, A., Lowy, D. & Scolnick, E. M. *J. Virol.* **44**, 509–519 (1982).
12. Shih, T., Stokes, P., Smythes, G., Dhar, R. & Oroszlan, S. *J. biol. Chem.* **257**, 11767–11773 (1982).
13. Ellis, R. W. *et al.* *Nature* **292**, 506–511 (1981).
14. Anderson, P. R. *et al.* *Cell* **26**, 129–134 (1981).
15. Dhar, R. *et al.* *Science* **217**, 934–937 (1982).
16. Lical, J. C. *et al.* *Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Stacey, D. W. & Allfrey, V. G. *Cell* **9**, 725–732 (1976).
18. Chang, E. H., Furth, M., Scolnick, E. & Lowy, D. *Nature* **297**, 479–483 (1982).
19. Stacey, D. W. & Allfrey, V. G. *J. Cell Biol.* **75**, 807–817 (1977).
20. Stacey, D. W. in *Introduction of Macromolecules into Viable Mammalian Cells* (eds. Baserga, R., Croce, C. & Rovera, G.) 125–134 (Liss, New York, 1980).
21. Mercer, W. E., Avignolo, C. & Baserga, R. *Molec. cell. Biol.* **4**, 276–281 (1984).
22. Stacey, D. W. *Meth. Enzym.* **79**, 76–88 (1981).

Expression of a bacterial gene in plants by using a viral vector

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Several properties of the cauliflower mosaic virus (CaMV) indicate that it could provide a useful vector for gene transfer in higher plants: (1) it has a relatively small double-stranded genome that can be easily manipulated *in vitro*^{1–3}; (2) cloned viral DNA is infectious when rubbed onto healthy leaves^{4,5}; (3) virus spreads throughout the plant and can be found in most cells at high copy number. Two regions of the CaMV genome—open reading frames (ORFs) II and VII—do not seem to be essential for infection, as both can be either deleted or expanded by small inserts of foreign DNA^{6–8}. No functional genes have yet been introduced into these ORFs. Here we report the replacement of CaMV ORF II by the R67 plasmid-encoded dihydrofolate reductase (DHFR) gene; this gene (*dhfr*) confers resistance to methotrexate in *Escherichia coli*. The chimaeric viral DNA can be stably propagated in turnip plants and the *dhfr* gene is expressed, producing a functional enzyme.

The CaMV ORFs, with the exception of ORF VI, are closely clustered on one strand of the DNA. Two virus-specific polyadenylated RNA species accumulate in infected cells^{9–11}; one of these is a 19S RNA that encodes the ORF VI gene product while the other is a 35S RNA, slightly larger than the full-length genome. It has been proposed that the tight arrangement of the CaMV ORFs is a prerequisite for the virus translation mechanism that may use a polycistronic messenger RNA (K. Sieg and B.G., in preparation). Therefore, we kept the distances between termination and initiation codons of ORFs in the CaMV constructions to a minimum. Figure 1a outlines how the *dhfr* gene, isolated from the plasmid vector pHG (ref. 12), was manipulated to remove noncoding sequences by two successive treatments

with the exonuclease *Bal31*. Deletions removing the entire upstream region of the initiation codon ATG were selected directly by placing an *EcoRI* linker in front of the ATG (followed by a G in the *dhfr* sequence¹³). This formed an *NcoI* site (CCATGG) which greatly facilitated plasmid screening.

The construction of two CaMV expression vectors is shown in Fig. 1b. The plasmid pCa20-Ball was used as the CaMV recipient for the *dhfr* gene in the first construction. In this plasmid, derived from strain CM4-184, ORF II is deleted except for the first five codons and the stop codon (K. Sieg and B.G., unpublished results). A unique *XhoI* site is present immediately in front of this stop codon. The *XhoI*–*SalI* fragment from pJP91 (Fig. 1a) was inserted into the *XhoI* site of pCa20-Ball to produce pCa-NB1, in which the *dhfr* sequence is inserted in the proper orientation (Fig. 1b). In the second construction, we further reduced the distance between the end of ORF I and the beginning of the *dhfr* gene by completely deleting ORF II from pCa20-Ball and inserting into the resulting plasmid, pCa-BB1, the *XhoI*–*SalI* fragment from pNC4X (Fig. 1). The resulting vector, pCa-NB2, has only 9 base pairs (bp) between the stop codon of ORF I and the initiation codon of the *dhfr* gene. As in pCa-NB1, only 1 bp remains between the *dhfr* termination codon and the ORF III initiation codon (Fig. 1b).

Turnip plants (*Brassica rapa*, cv. 'Just Right') were inoculated with cloned DNA excised from the bacterial vector. Symptoms of infection developed after the same period as for control plants infected with CaMV sequences from parents pCa20-Ball and pCa-BB1, which suggests that the presence of the *dhfr* gene was not detrimental to virus replication. Proof that the gene had been retained in the viral genome was obtained by isolating virus particles from systemically infected leaves. Figure 2 (lanes 2, 6) shows that when DNA prepared from these viruses was digested with *XhoI* and *ClaI*, the DNA fragment containing the *dhfr* gene (arrows) was present in each case in the expected molar ratio, indicating that the foreign DNA was maintained in the respective viral genomes. Similar results were obtained after two and three cycles of infection with Ca-NB2 (Fig. 2, lanes 7, 8). However, the intensity of the *dhfr*-containing band in Ca-NB1 was significantly reduced and minor bands appeared when virus was isolated from plants after a second and third cycle of infection (Fig. 2, lanes 3, 4), suggesting that some DNA rearrangements had occurred within the *XhoI*–*ClaI* fragment containing the *dhfr* sequence.

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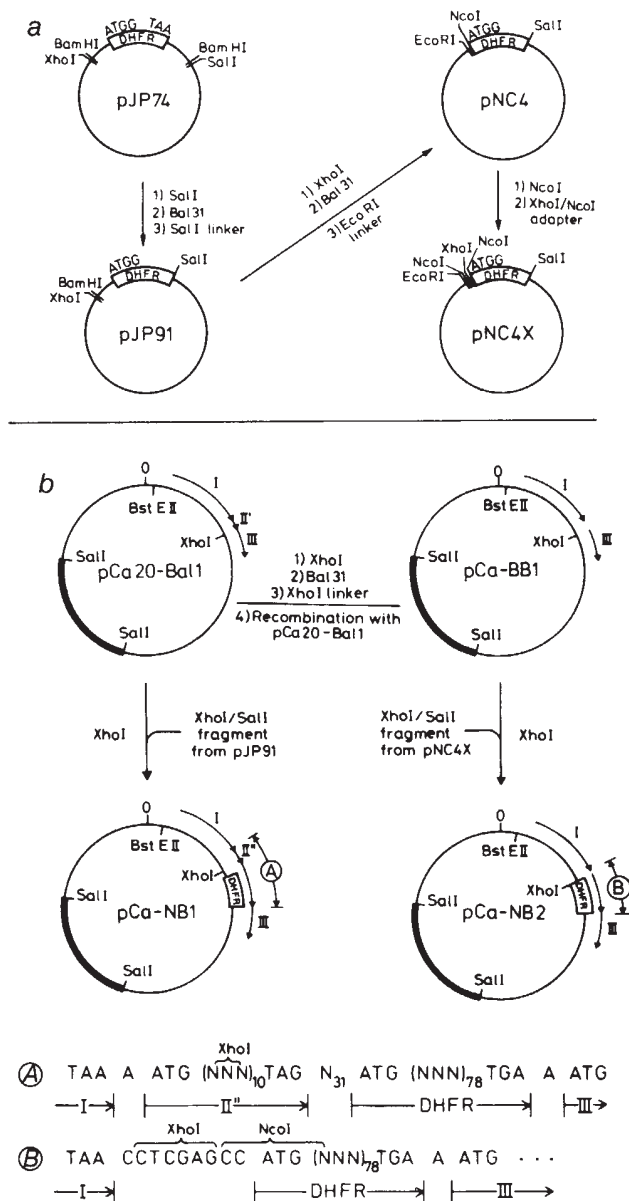


Fig. 2 Electrophoretic analysis of chimaeric CaMV DNA. *Brassica rapa* cv. 'Just Right' plants were inoculated with cloned CaMV DNA that had been digested with *SalI* (ref. 5). Each plant was infected with 4 µg of DNA and the first symptoms of infection usually appeared within 10–14 days. DNA of the viral progeny was prepared from individual plants¹⁷ and used to inoculate new plants. Viral DNA from each cycle of infection was digested with a combination of *XhoI* and *ClaI* restriction enzymes and electrophoresed on a 1.2% agarose gel. The DNA fragments carrying the *dhfr* sequence (arrows) have been identified by hybridization with the *NcoI*–*SalI* *dhfr* fragment from pNC4 (not shown). Lane 1, pCa-NB1 plasmid DNA; lanes 2–4, Ca-NB1 viral DNA from 1st, 2nd and 3rd cycles of infection, respectively; lane 5, pCa-NB2 plasmid DNA; lanes 6–8, Ca-NB2 viral DNA from 1st, 2nd and 3rd cycles of infection.

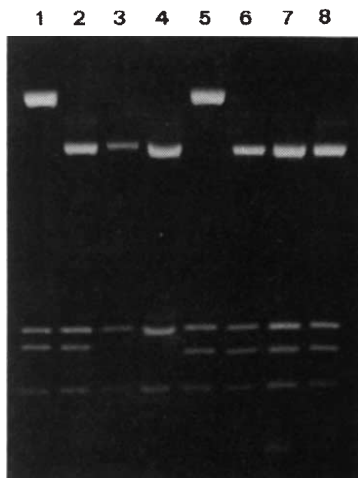


Fig. 1 Construction of CaMV vectors directing the synthesis of a methotrexate-resistant dihydrofolate reductase. **a**, Construction of the R67-*dhfr* gene derivatives. **b**, Construction of the CaMV vectors.

Methods: **a**, The *Bam*HI fragment from the plasmid pHG (ref. 12) which contains the entire R67-*dhfr* gene sequence was inserted into pUC8X (ref. 13). 20 µg of the resulting plasmid (pJP74) were digested with *SalI* and treated with 2 U of the *Bal*31 exonuclease (Biolabs) for 1–8 min in 100 µl of 12 mM MgCl₂, 12 mM CaCl₂, 200 mM NaCl, 1 mM EDTA, 240 µg ml⁻¹ bovine serum albumin (BSA) and 20 mM Tris-HCl, pH 8.0 at 30 °C. The *Bal*31-treated molecules were incubated with DNA polymerase (Klenow fragment) and the four deoxynucleoside triphosphates (at 10 mM each) to fill in the ends. *SalI* linkers were added and, following ligation and transformation, plasmids were screened for deletions ending near the *dhfr* gene stop codon by polyacrylamide gel electrophoresis and nucleotide sequencing¹⁶. One clone (pJP91) lacking the stop codon plus 5 bp of coding sequence was digested with *XhoI* and again treated with exonuclease *Bal*31 (1–4 min) as above. After ligation in the presence of *EcoRI* linkers, plasmids were screened for the presence of a *NcoI* site (see text). Plasmid pNC4, which contained all the region upstream from the ATG deleted, was digested with *NcoI* and religated in the presence of a *XhoI*–*NcoI* adapter to yield pNC4X. Transformations were performed using *E. coli* strain DH1. ATGG refers to the first four nucleotides of the *dhfr* gene and TAA to the stop codon. **b**, The *XhoI*–*SalI* fragment from pJP91 was isolated from a 6% polyacrylamide gel and ligated into the *XhoI* site of pCa20-Bal1. Plasmid pCa-NB1 carries the *dhfr* sequence in the proper orientation relative to the other CaMV open reading frames. To remove ORF II completely from the CaMV genome, pCa20-Bal1 was digested with *XhoI*, treated with *Bal*31 and recircularized in the presence of *XhoI* linkers. The resulting plasmid (pCa-Bal2, not shown) having the whole ORF II plus 1 bp removed was selected by DNA sequencing. To reconstitute the initiation codon of ORF III which had been deleted by the exonuclease treatment, the small *BSTEII*–*XhoI* fragment from pCa-Bal2 was isolated from a 0.6% agarose gel and ligated to the large *BSTEII*–*XhoI* fragment of pCa20-Bal1. The resulting plasmid (pCa-BB1) was digested with *XhoI* for ligation with the *XhoI*–*SalI* *dhfr* fragment from pNC4X. pCa-NB2 carries the *dhfr* gene in the proper orientation. The nucleotide sequence of the CaMV–*dhfr* junctions for the two constructions are shown in A and B. Note that the last two codons of the *dhfr* gene have been changed and an extra amino acid is now encoded at the 3' end of the molecule due to the presence of a *SalI* linker and to the *SalI*–*XhoI* fusion. Small modifications at this end of the protein do not affect its activity¹³. The size of the inserted *dhfr* sequence is 234 bp. Roman numerals indicate the position of the open reading frames. II' and II'' are short ORFs that include the first five codons of ORF II. The solid box represents the bacterial vector pUC8.

The reason for the instability of the inserted sequence in Ca-NB1 is unclear. We presume that the long intergenic sequence upstream from the initiation codon of the *dhfr* gene (Fig. 1b, A), present neither in wild-type CaMV nor in pCa-NB2, interferes with the translation of the viral transcript. Similar constructions in which a long intergenic distance was present at either the 5' or 3' extremity of the *dhfr* gene were even less stable than Ca-NB1 (data not shown).

Expression of the *dhfr* gene in turnip plants was monitored using a specific anti-DHFR antiserum. Total proteins, extracted from infected and uninfected tissues, were separated by electrophoresis on a 15% SDS-polyacrylamide gel, transferred to nitrocellulose paper and reacted with the anti-R67-*dhfr* serum. A protein present in the samples derived from pCa-NB1 and pCa-NB2 gave rise to an antigenic reaction (Fig. 3, lanes 7, 8), but no reaction was detected with the uninfected and pCa-BB1-derived samples (lanes 5, 6). Furthermore, the protein detected in lanes 7 and 8 of Fig. 3 co-migrated with R67-*dhfr* purified from *E. coli* (lane 9). This shows that the DHFR protein encoded by the two chimaeric viral genomes Ca-NB1 and Ca-NB2 has

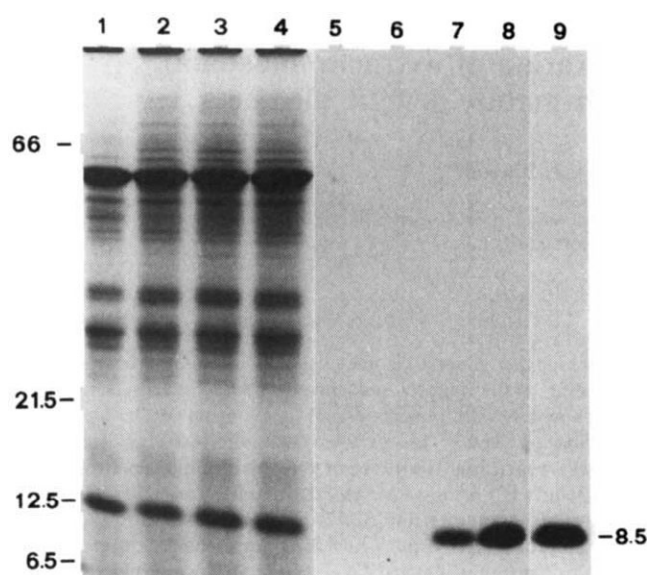


Fig. 3 Synthesis of R67-dhfr protein in infected plants. Leaves (2.5 g) were collected 4 weeks after inoculation with cloned DNA, washed, ground in liquid nitrogen and homogenized in 10 ml buffer (2% SDS, 300 mM 2-mercaptoethanol, 10 mM phenylmethylsulphonyl fluoride, 50 mM Tris-HCl, pH 7.0). After filtration through four layers of cheesecloth and two layers of Miracloth, 5 volumes of acetone were added and the proteins precipitated overnight at -20°C . Protein pellets were resuspended in 1 vol. electrophoresis sample buffer¹⁸ and 50 μg of each protein were applied in duplicate on a 15% SDS-polyacrylamide gel. One set was stained with Coomassie brilliant blue R (lanes 1–4) and the other was transferred electrophoretically¹⁹ onto nitrocellulose paper. The paper was equilibrated for 3 h in 200 ml BSA buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.2% Triton X-100, 3% BSA) and incubated with antibody in 20 ml of the same buffer overnight. The blot was washed five times in 200 ml of 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100 and incubated with ^{125}I -protein A (1 μCi in 15 ml BSA buffer; Amersham). After washing, the blot was autoradiographed on an X-ray film (lanes 5–8). Antiserum was prepared by immunizing female New Zealand White rabbits with purified R67-dhfr²⁰. Lanes 1, 5, proteins from uninfected plants; lanes 2, 6, from plants infected with pCa-BB1; lanes 3, 7, from plants infected with pCa-NB1; lanes 4, 8, from plants infected with pCa-NB2. Lane 9 contains 100 ng of purified R67-dhfr protein that had been loaded on the same gel and reacted with the antibody.

been synthesized in turnip plants. By comparing the intensity of the bands in lanes 8 and 9 of Fig. 3, and assuming that the DHFR protein in both lanes has been transferred to nitrocellulose with equal efficiency, one can estimate that pCa-NB2-infected leaves contain approximately 8 μg of DHFR per g fresh tissue. The reduced level of expression of *dhfr* in Ca-NB1-infected tissues (lane 7) could be related to the instability of the *dhfr* gene in this construction.

Dihydrofolate reductase catalyses the reduction of dihydrofolate to tetrahydrofolate. Inhibition of this reaction by antifolate compounds, such as methotrexate, results in impaired synthesis of RNA and DNA¹⁴. However, the dihydrofolate reductase encoded by plasmid R67 is practically insensitive to methotrexate¹⁵. It was therefore possible to test for the presence of the R67-dhfr activity in turnip plants infected with pCa-NB1 and pCa-NB2 by measuring the effect of methotrexate on DNA synthesis. Four weeks after inoculation with cloned DNA, systemically infected leaves were collected and incubated in culture medium containing free ^{32}P -phosphate in the presence and absence of the drug. Total DNA was then prepared and incor-

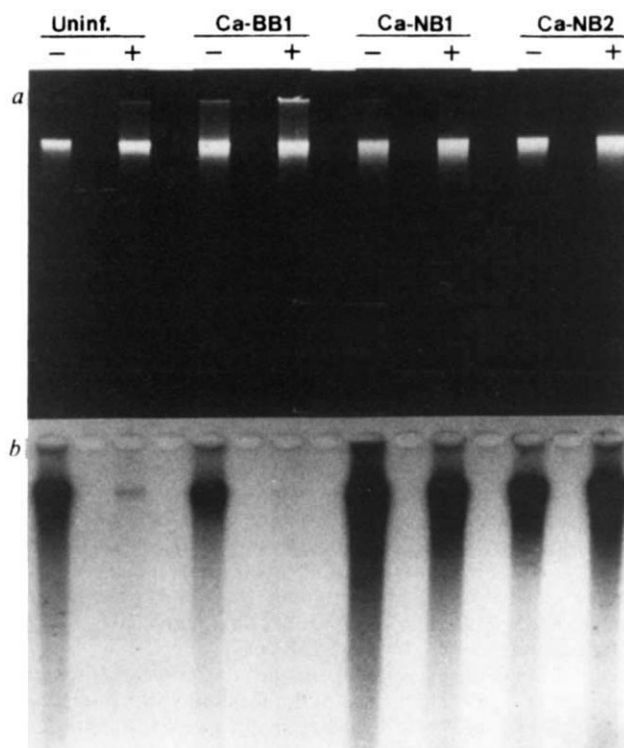


Fig. 4 Assay of methotrexate-resistant activity in plant cells. *a*, DNA stained with ethidium bromide; *b*, autoradiography of the gel shown in *a*. + and – refer to the presence or absence, respectively, of methotrexate in the culture medium.

Methods: Young, fully expanded leaves were collected, washed and surface-sterilized for 30 min in 0.5% $\text{Ca}(\text{OCl})_2$, followed by thorough washing in sterile water. Leaves were cut into segments and immersed in filter-sterilized NN69 culture medium²¹ (0.5 mg l^{-1} NAA; 0.25 mg l^{-1} 2, 4D; 0.1 mg l^{-1} BAP, pH 5.8) containing no phosphate salts. Duplicate samples contained methotrexate (Sigma) at a concentration of 0.1 $\mu\text{g ml}^{-1}$. Cultures were incubated at 26°C in the dark on a gyratory shaker for 22 h. Medium was removed and replaced by 5 ml of fresh medium (containing methotrexate when required) and 35 μCi of ^{32}P -orthophosphate (Amersham). The cultures were incubated with shaking for 22 h, the medium was removed and the leaf tissue washed three times in 15 ml phosphate buffer before freezing in liquid nitrogen. The tissue was homogenized in 0.5 ml of 50 mM NaCl, 10 mM EDTA, 1% sarkosyl, 10 mM Tris-HCl, pH 7.5, and extracted twice with 1 vol. of phenol. Nucleic acids were precipitated with isopropanol and the pellet redissolved in 50 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) containing 20 $\mu\text{g ml}^{-1}$ RNase. After 1 h at 37°C , 450 μl of 200 $\mu\text{g ml}^{-1}$ proteinase K in 0.1% SDS were added for another hour at 37°C , followed by phenol extractions and isopropanol precipitations. Pellets were resuspended in 50 μl TE buffer and DNA was electrophoresed on a 1% agarose gel.

poration of ^{32}P -phosphate evaluated. Controls were prepared from leaves from uninfected plants and from plants infected with the CaMV vector lacking the *dhfr* gene (pCa-BB1).

Figure 4b is an autoradiograph of a gel (Fig. 4a) containing equivalent amounts of DNA extracted from uninfected and infected tissues. It can be seen that leaves infected with *dhfr*-containing chimaeric viruses (Ca-NB1 and Ca-NB2) incorporated ^{32}P into DNA both in the presence and absence of methotrexate, whereas DNA synthesis in uninfected leaves ('Uninf.') or in those carrying control virus ('Ca-BB1') was blocked by methotrexate. This indicates that the methotrexate-insensitive DHFR enzyme encoded by Ca-NB1 and Ca-NB2 is active in turnip plants. The relative radioactivity in each labelled

DNA was determined in three experiments and in general, the incorporation of ^{32}P -phosphate into DNA in the presence of methotrexate was 12–21-fold higher in pCa-NB1 ($72 \pm 14\%$) and pCa-NB2 ($84 \pm 4\%$) infected tissues than in control tissues ($3.9 \pm 0.5\%$ and $5.9 \pm 5\%$ in uninfected and Ca-BB1-infected tissues, respectively).

Phenotypic expression of the *dhfr* gene in turnip plants was evaluated by spraying plants with a methotrexate solution. Eight to ten days later, plants infected with viruses containing the *dhfr* gene were little affected whereas uninfected plants and plants infected with pCa-BB1 showed prominent symptoms of senescence (not shown).

The fact that the complete ORF II of CaMV can be deleted and replaced by a foreign gene without significantly affecting the infectivity and replication of the virus indicates that no important transcriptional or translational signal sequences are present in this region. Further work is required to show whether DNA sequences larger than the relatively small R67-dhfr gene (234 bp) can be maintained in the viral genome. This should be possible by using all the space provided by the ORF II deletion (470 bp) and by taking advantage of a possible overpackaging capacity of CaMV particles⁷. Additional space in CaMV DNA could also be obtained by combining the ORF II deletion with a large deletion made in ORF VII (L. Dixon and T.H., unpublished results).

The successful expression of the bacterial *dhfr* coding sequence in turnip demonstrates the feasibility of using a viral vector to propagate and express foreign genes in plants. This type of vector should be particularly convenient for mediating the rapid introduction of foreign genes into whole plants, and also for the expression of cDNA sequences. The high copy number and level of expression of a CaMV vector could lead to synthesis of relatively large amounts of a protein in infected cells. It should now be possible to use the CaMV-*dhfr* constructions, in combination with the rapid and sensitive assay for DHFR activity, to improve the usefulness of CaMV as a vector. For example, one could screen for new host plants on the basis of the expression of drug resistance rather than solely on the appearance of symptoms. Similarly, less virulent CaMV strains might be isolated by introducing mutations in the chimaeric genome and by screening for those which eliminate symptoms without affecting viral replication.

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1. Franck, A., Guillely, H., Jonard, G., Richards, K. & Hirth, L. *Cell* **21**, 285–294 (1980).
2. Gardner, R. C. *et al. Nucleic Acids Res.* **9**, 2871–2888 (1981).
3. Bárány, E., Guillely, H., Jonard, G. & Richards, K. *Gene* **19**, 239–249 (1982).
4. Howell, S. H., Walker, L. L. & Dudley, R. K. *Science* **208**, 1265–1267 (1980).
5. Lebeurier, G., Hirth, L., Hohn, T. & Hohn, B. *Gene* **12**, 139–146 (1980).
6. Howell, S. H., Walker, L. L. & Walden, R. M. *Nature* **293**, 483–486 (1981).
7. Gronenborn, B., Gardner, R. C., Schaefer, S. & Shepherd, R. J. *Nature* **294**, 773–776 (1981).
8. Dixon, L. K., Koenig, I. & Hohn, T. *Gene* **25**, 189–199 (1983).
9. Odell, J. T., Dudley, R. K. & Howell, S. H. *Virology* **111**, 377–385 (1981).
10. Guillely, H., Dudley, R. K., Jonard, G., Bárány, E. & Richards, K. E. *Cell* **30**, 763–773 (1982).
11. Covey, S. N. & Hull, R. *Virology* **111**, 463–474 (1981).
12. O'Hare, K., Benoist, C. & Breathnach, R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1527–1531 (1981).
13. Brisson, N. & Hohn, T. *Gene* **28**, 265–269 (1984).
14. Blakley, R. L. *The Biochemistry of Folic Acid and Related Pteridins*, 139–187 (North-Holland, Amsterdam, 1969).
15. Pattishall, K. H., Acar, J., Burchall, J. J., Goldstein, F. W. & Harvey, R. J. *J. biol. Chem.* **252**, 2319–2323 (1977).
16. Maxam, A. M. & Gilbert, W. *Methods Enzym.* **65**, 499–560 (1980).
17. Gardner, R. C. & Shepherd, R. J. *Virology* **106**, 159–161 (1980).
18. Laemmli, U. K. *Nature new Biol.* **227**, 680–685 (1970).
19. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. USA* **76**, 4350–4354 (1979).
20. Fling, M. E. & Ellwell, L. P. *J. Bact.* **141**, 779–785 (1980).
21. Nitsch, J. P. & Nitsch, C. *Science* **163**, 83–87 (1969).

Role of reverse transcription in the generation of extrachromosomal *copia* mobile genetic elements

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The *Drosophila* genetic element *copia* is one of the best studied eukaryotic transposable sequences. *Copia* shares structural features with a wide variety of mobile elements in *Drosophila*^{1,2}, *Lepidoptera*³, yeast^{4,5} and vertebrates^{1,2}, the last class being the retrovirus proviruses. Furthermore, retrovirus-like particles containing *copia* RNA have been isolated from *Drosophila* cells⁶ and extrachromosomal circular *copias* with structures closely resembling circular retrovirus proviruses have been isolated and cloned^{7,8}. Therefore, *copia*-like elements and retroviruses may be members of a class of mobile genetic elements existing throughout the eukaryotic kingdom. Consequently, there has been speculation that retroviruses evolved from transposable elements^{7–9} and, conversely, that *copia*-like elements transpose as retroviruses or retrovirus-like particles^{6–8}. To date, however, there has been no demonstration that *copia* RNA is reverse transcribed into *copia* DNA. The present report describes the isolation of linear extrachromosomal *copias* whose structure closely resembles the analogous retrovirus provirus linears and whose synthesis is unaffected by inhibitors of the cellular DNA polymerase responsible for chromosomal DNA replication.

Using a modification of the technique of Flavell and Ish-Horowicz⁷ for isolating circular extrachromosomal *copias*, I have detected linear extrachromosomal *copias* (Fig. 1a). The molecules are of identical size to a linearized complete *copia* circle containing two long terminal repeats (LTRs). The major extrachromosomal *copia* circle contains only one LTR but very low levels of 1-LTR linears were present, suggesting that the bulk of the linear extrachromosomal *copias* is not derived by random degradation during the isolation procedure. Furthermore, 2-LTR *copia* linears possess a non-permuted structure. Digestion with *Eco*RI, which cuts the centre of genomic *copias* at two closely spaced sites (Fig. 1b), yields two fragments of 2.3 and 2.4 kilobases (kb), consistent with the structure shown in Fig. 1d. Circular *copias* are effectively linearized by this procedure and contaminating genomic *copias* generate a smear of fragments greater than 2.3 kb due to random *Eco*RI sites outside the elements. Digestion of linear *copias* with *Pvu*II and *Pst*I (which each cleave *copia* at one site) gave the expected end fragments (Fig. 1c). Therefore, *copia* linears have a non-permuted structure closely resembling linear retrovirus proviruses.

To address the question of whether extrachromosomal linear *copias* are derived by reverse transcription, I have analysed the uptake of 5-bromodeoxyuridine (BUdR) into these molecules. This compound has been used to probe the mechanism of synthesis of retrovirus proviruses¹⁰. Non-conservative synthesis of provirus DNA by reverse transcription yields proviral DNA labelled on both strands (HH DNA) whereas semiconservative replication of chromosomal DNA labels only one strand (HL DNA) after one round of replication. These DNAs can be isolated from each other and from non-replicated DNA (LL DNA) by CsCl density gradient ultracentrifugation¹⁰.

Extrachromosomal DNA was isolated from *Drosophila* cells by Hirt extraction¹¹ and CsCl isopycnic ultracentrifugation to resolve HH, HL and LL DNAs. Extrachromosomal *copias* were detected by agarose gel electrophoresis and Southern filter

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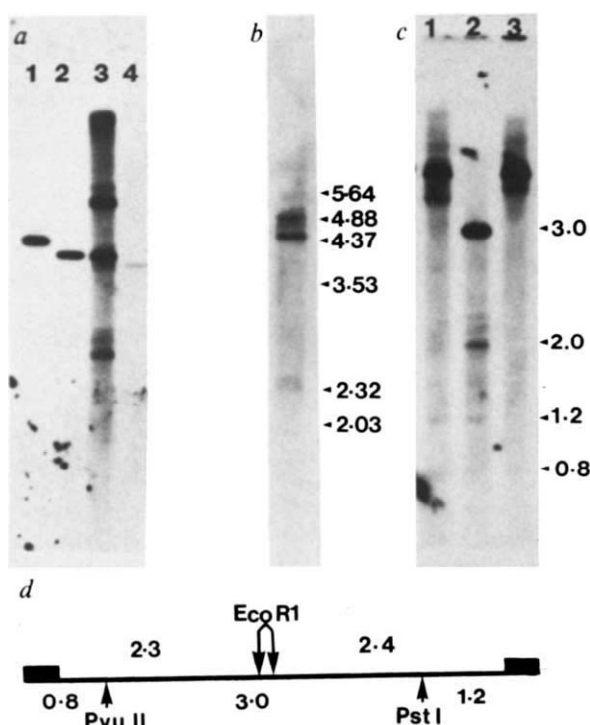
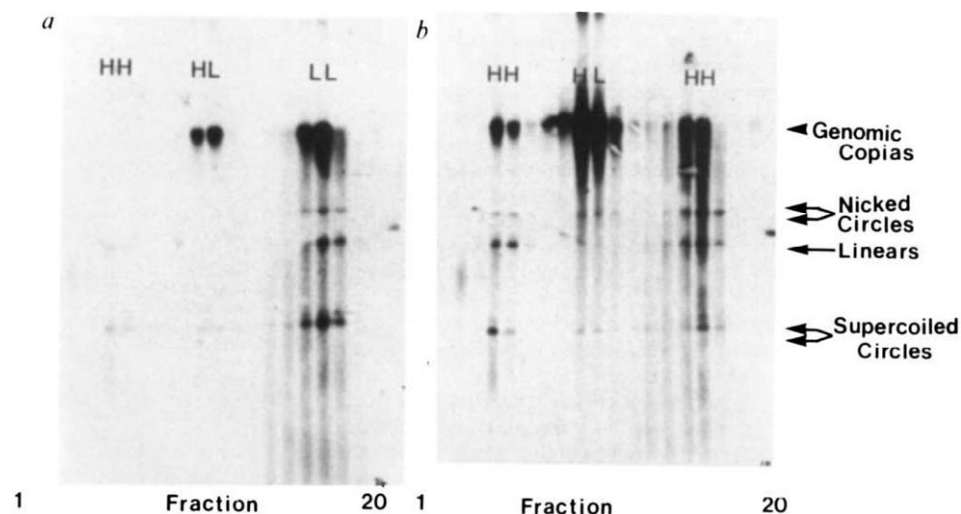


Fig. 1 Characterization of extrachromosomal *copia* linear DNA. A sample of extrachromosomal *copia* DNA was purified by Hirt extraction¹¹ and CsCl isopycnic ultracentrifugation, then assayed for *copia* sequences by Southern blotting^{7,12}. **a**, Lane 1, 50 pg of a *Msp*I restriction fragment of cDM2056 containing an intact genomic *copia* element together with 190 base pairs of flanking *Drosophila* DNA⁷. Lane 2, a linearized 2-LTR *copia* circle (pBB1) digested with *Hind*III⁷. Lane 3, extrachromosomal *Drosophila* cultured cell DNA. Lane 4, a linearized 1-LTR *copia* circle (pBB3) digested with *Hind*III⁷. **b**, Digestion of *copia* extrachromosomal DNA with *Eco*RI. A DNA sample identical to that used in **a**, lane 3 was digested with *Eco*RI and assayed for *copia*-specific sequences. The mobilities of marker *Hind*III-digested λ DNA fragments are shown. **c**, Digestion of *copia* extrachromosomal DNA with *Pst*I and *Pvu*II. A DNA sample identical to that used above was digested with *Pst*I (lane 1), *Pvu*II (lane 3) or both enzymes (lane 2). The sizes of the bands were calculated from the mobilities of parallel marker DNA fragments. **d**, *Eco*RI, *Pvu*II and *Pst*I restriction map of a putative extrachromosomal linear *copia* element. The LTRs are shown (■).

Fig. 2 Incorporation of bromodeoxyuridine into extrachromosomal *copia* elements. 5-Bromodeoxyuridine (100 μ g ml⁻¹) was added to exponentially growing Kc *Drosophila* cultured cells²⁴ (10⁸ cells). Extrachromosomal DNA was isolated from the cells by the method of Hirt¹¹ at 24 h (**a**) or 42 h (**b**) after BUdR addition. The enriched extrachromosomal DNA sample was subjected to CsCl isopycnic ultracentrifugation (10 ml 10 mM Tris-HCl (pH 7.5), 1 mM EDTA plus 13.71 g CsCl in a Beckman 50Ti rotor at 35,000 r.p.m. for 48 h). Nucleic acids were recovered from the fractionated gradient by ethanol precipitation. 50% of each fraction was assayed for *copia*-specific sequences by agarose gel electrophoresis (0.8% agarose in Tris-borate pH 8.3), Southern blotting and probing with ³²P-nick-translated *copia* DNA. The washed filter was exposed to Fuji RX film with an intensifying screen at -70°C for 72 h. Fraction 1 is the bottom of the gradient, fraction 20 contains 50 pg of a linear genomic 5.1-kb *copia* element (see Fig. 1 legend)⁷. The locations of HH, HL and LL genomic DNAs in the gradients are shown (see text). The electrophoretic mobilities of high molecular weight genomic DNA, nicked circular 1-LTR and 2-LTR *copia* DNAs, extrachromosomal linear *copia*s and 1-LTR and 2-LTR supercoiled *copia* circles are shown^{7,8}.



hybridization¹². Figure 2 shows the incorporation of BUdR into extrachromosomal *copia*s at varying times after addition to the medium. HH, HL and LL DNAs are well resolved, as are the linear 2-LTR *copia*s and supercoiled and nicked 1-LTR and 2-LTR circular *copia*s, together with contaminating genomic DNA.

All three types of HH extrachromosomal *copia*s (2-LTR linears and 1-LTR and 2-LTR circles) arise much more rapidly than the corresponding HH genomic DNA. Figure 2a shows that after 24 h significant levels of all three HH extrachromosomal *copia*s have undergone two cycles of semiconservative replication (as judged by the high molecular weight genomic *copia* DNA signal in Fig. 2a). After 42 h, roughly 50% of the extrachromosomal *copia*s are in the HH fraction but the amount of HH genomic *copia* also has increased (Fig. 2b). By visual comparison of the HH to HL ratio of extrachromosomal *copia*s with the corresponding ratio for genomic *copia*s in several different exposures of the autoradiograms in Fig. 1, I conclude that all three types of HH *copia* extrachromosomal DNA arise approximately 10 times more rapidly than HH genomic DNA.

These data show that extrachromosomal HH *copia* DNA accumulates more rapidly than HH genomic *copia*s and are consistent with the proposition that extrachromosomal *copia*s are generated by reverse transcription. However, they do not exclude the possibility that some circular *copia*s replicate semi-conservatively at a higher rate than genomic DNA. To resolve this, the endogenous background of semiconservative DNA replication was lowered by use of the DNA polymerase α inhibitor aphidicholin (see ref. 13 for review). As aphidicholin does not inhibit vertebrate reverse transcriptase^{14,15}, a similar experiment to that shown in Fig. 2 was performed in the presence of this inhibitor (Fig. 3). In this case, accurate quantitative determination of the inhibition of semiconservative replication was made by isolating chromosomal DNAs from the Hirt pellets and subjecting them to isopycnic ultracentrifugation and Southern dot-blot analysis.

Comparison of amounts of HH, HL and LL genomic *copia*s accumulated after a 24-h BUdR labelling in the presence and absence of aphidicholin (Fig. 3a) indicated that aphidicholin inhibited the incorporation of BUdR into HL genomic DNA by ~65%. If HH extrachromosomal *copia*s were generated by two rounds of semiconservative replication, this would be reflected as a compound of two successive 65% inhibitions such that 12% of the control level of HH extrachromosomal *copia* DNA would be expected. Figure 3b, c demonstrates that there is no

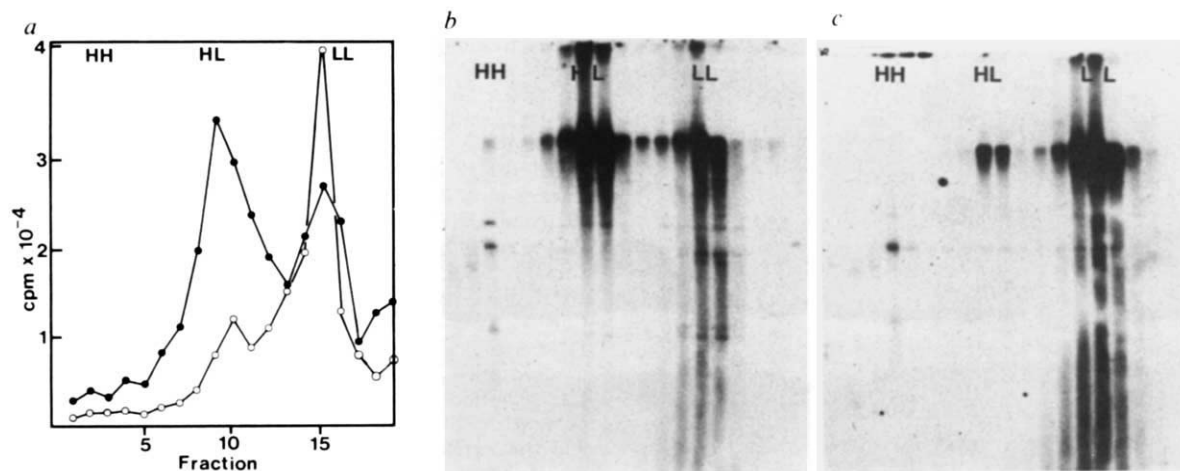


Fig. 3 The effect of aphidicholin on BUdR incorporation into extrachromosomal *copias*. A similar experiment to that described in Fig. 2 was performed with the modification that aphidicholin was added to one set of dishes ($50 \mu\text{g ml}^{-1}$, 10^8 cells) 6 h before BUdR addition. This treatment resulted in negligible cell lethality in control experiments and the cultures could be rescued by medium replacement (data not shown). Hirt extraction was performed 24 h after addition of BUdR¹¹. *a*, Genomic DNA was isolated from the Hirt pellets of the above experiments and assayed for *copias* sequences in HH, HL and LL DNAs by CsCl ultracentrifugation followed by dot-blot analysis. $10 \mu\text{l}$ of each gradient fraction was spotted direct onto dry nitrocellulose, the filter was placed in 0.5 M NaOH , 1 M NaCl for 5 min followed by $1 \text{ M Tris-HCl pH } 7.5$, 3 M NaCl for 15 min. The filter was baked and probed with ^{32}P -labelled *copias* DNA ($10^8 \text{ c.p.m. per } \mu\text{g}$) as before, the individual fraction spots were cut out and assayed for Cerenkov radiation in a scintillation counter. ●, Minus aphidicholin; ○, plus aphidicholin. The gradient locations of HH, HL and LL DNAs are shown. *b*, Hirt supernatants from the minus aphidicholin control were treated as for Fig. 2. *c*, Hirt supernatants from the aphidicholin-treated cells were assayed for extrachromosomal *copias* for Fig. 2.

detectable inhibition of synthesis of HH *copias* linears in the presence of aphidicholin. However, the corresponding levels of both classes of HH *copias* circles are reduced about threefold by aphidicholin. Analogous experiments with retroviruses have given similar results^{14,15}, leading to the conclusion that DNA polymerase α may be involved in the maturation of circular proviruses, possibly in the circularization step(s).

Could linear extrachromosomal *copias* be synthesized by an alternative DNA-dependent DNA polymerase? The other cellular polymerases (β and γ) are resistant to aphidicholin but polymerase β is exclusively a DNA repair enzyme¹⁶ and polymerase γ is a mitochondrial replication enzyme¹⁶. I know of no report of viral DNA polymerases in *Drosophila*. Furthermore, viral DNA-dependent DNA polymerases such as those of adenoviruses and herpes viruses which replicate linear viral DNAs require structural features at the linear termini which are not found in *copias*¹⁷; indeed, all the structural features of *copias* point to a reverse transcription model for its replication. The only plausible enzyme is therefore a reverse transcriptase. Although it is possible that extrachromosomal *copias* are generated by a polymerase not associated with a virus-like particle, the most likely explanation for the data reported here and those of Shiba and Saigo⁶ is that *copias* can exist as a retrovirus-like entity analogous to the A-type particles of mice^{18,19}. In support of this conclusion, reverse transcriptase-like activity has been reported in *Drosophila* cultured cells²⁰ and in *Drosophila* retrovirus-like particles⁶.

Several experiments suggest that a proportion of circular *copias* can be derived by alternative mechanisms to reverse transcription. First, the sequence of two cloned 2-LTR circles at the LTR-LTR junctions suggests that these particular molecules were created by excision of genomic *copias*⁸. 1-LTR circles cannot be analysed in this way, but studies with other *copias*-like elements support the view that 1-LTR circles can be derived by genomic excision. LTR-LTR recombinative excision of genomic *copias*-like elements has been observed in yeast²¹ and mice²²; in the latter case the element is an ecotropic murine leukaemia retrovirus provirus. Second, *copias* circles may be derived by replication as plasmids. The HL *copias* circles seen in the present experiments (Figs 2, 3) could represent semicon-

servatively replicating *copias* circles, recently excised *copias* which replicated in the genome during the labelling period or HH *copias* circles which incorporated BUdR shortly after its addition to the cells (when the intracellular concentration of BUdR, and hence the density of the resultant DNA, was lower). The third explanation is unlikely as there is a discernible optimal density of these molecules, corresponding exactly to that of HL genomic DNA. These experiments do not resolve the first two possibilities, but I consider it likely that semiconservative replication is at least in part responsible for the synthesis of HL *copias* circles. *Copias*-based plasmid vectors are capable of replication in cultured *Drosophila* cells²³, although it is not known whether *copias* possesses the replication origin in these constructs. Such replication is presumably semiconservative.

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1. Rubin, G. M. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **45**, 619-628 (1981).
2. Varmus, H. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) (Academic, New York, 1982).
3. Miller, D. W. & Miller, L. K. *Nature* **299**, 562-564 (1982).
4. Farabaugh, P. J. & Fink, G. R. *Nature* **286**, 352-356 (1980).
5. Gafner, J. & Philippson, P. *Nature* **286**, 414-418 (1980).
6. Shiba, T. & Saigo, K. *Nature* **302**, 119-124 (1983).
7. Flavell, A. J. & Ish-Horowicz, D. *Nature* **292**, 591-595 (1981).
8. Flavell, A. J. & Ish-Horowicz, D. *Cell* **34**, 415-419 (1983).
9. Temin, H. M. *Cell* **21**, 599-600 (1980).
10. Varmus, H. E. & Shank, P. R. *J. Virol.* **18**, 567-573 (1976).
11. Hirt, B. *J. molec. Biol.* **26**, 365-369 (1967).
12. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
13. Jacob, S. T. (ed.) *Enzymes of Nucleic Acid Synthesis and Modification* Vol. 1, 54-55 (CRC Press, Boca Raton, Florida, 1983).
14. Hagino-Yamagishi, K., Kano, K. & Mano, Y. *Biochem. biophys. Res. Commun.* **102**, 1372-1378 (1981).
15. Hsu, T. W. & Taylor, J. M. *J. Virol.* **44**, 493-498 (1982).
16. Hubscher, U., Kuenzle, C. C. & Spadari, S. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2316-2320 (1979).
17. Toozé, J. (ed.) *DNA Tumour Viruses. Molecular Biology of Tumour Viruses* 2nd edn (Cold Spring Harbor Laboratory, New York, 1981).
18. Yang, S. S. & Wivel, N. A. *J. Virol.* **13**, 712-720 (1974).
19. Kuff, E. L. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 1992-1996 (1983).
20. Heine, C. W., Kelly, D. C. & Avery, R. J. *J. gen. Virol.* **49**, 385-395 (1980).
21. Fink, G., Farabaugh, P., Roeder, G. & Chaleff, D. *Cold Spring Harb. Symp. quant. Biol.* **45**, 575-580 (1980).
22. Copeland, N. G., Hutchison, K. W. & Jenkins, N. A. *Cell* **33**, 379-387 (1983).
23. Sinclair, J. H., Sang, J. H., Burke, J. F. & Ish-Horowicz, D. *Nature* **306**, 198-200 (1983).
24. Eschallier, G. & Ohanessian, A. *In Vitro* **6**, 162-172 (1970).

Round the world of climatology

T. M. L. Wigley

The Coevolution of Climate and Life. By Stephen H. Schneider and Randi Londer. *Sierra Club Books: 1984. Pp. 563. \$25, £21.75.*

A GOOD book should require no lengthy accolade. Schneider and Londer's book is good, and I can summarize it no better than do the authors in their opening sentence.

The Coevolution of Climate and Life explores the earth's climate history and some possible scenarios for its future; the mechanisms of climate change; and the political and ethical implications of the discoveries of climatologists.

These are ambitious aims. They are achieved here in eminently readable prose, the text brimming also with facts and details. At times the reader may be almost overwhelmed by the rate at which information is introduced — but this will serve only to excite and stimulate. Perhaps the most refreshing aspect of this book is its critical attitude. This is no boring review of the field; rather it is a true synthesis,

discussing topics in a broad framework, gently demolishing many of the idle speculations which have crept into the literature, and giving the reader fresh insights.

Who, then, is this book directed towards? It is clearly meant not only for the lay-person, but also for a wide spectrum of decision-makers in politics, science and industry. It is absorbing, comprehensive and so authoritative that it should find wide use as a textbook in climatology or palaeoclimatology. The references are extensive and provide a well-selected guide to the modern literature of that diverse variety of fields which impinge on climatology. □

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Making drugs (and soaking the poor?)

Carl Djerassi

The Pharmaceutical Industry and Dependency in the Third World.

By Gary Gereffi.

Princeton University Press: 1983. Pp. 291. Hbk \$25, £25; pbk \$9.95, £7.70.

I DOUBT whether an accurate and all-encompassing account of the pharmaceutical industry and Third World countries can be written by one author. Certainly, Gary Gereffi is not that person. Ideally the author would combine the expertise of a development economist (Gereffi fits that requirement) with practical experience of the management of a large pharmaceutical company (Gereffi has none), and be a chemist (I shall not cite the many mistakes to illustrate that chemistry is not Gereffi's forte). This last requirement is imposed by Gereffi's choice of the specific illustration to support his thesis — namely, the steroid industry in Mexico. This leads to the second reason why this book is historically valuable but almost useless from the standpoint of policy. I can think of no worse example to discuss the dependency of Third World countries in the manufacture and distribution of pharmaceuticals than the rise and fall of Mexico's steroid industry, which is completely *sui generis*.

The first part of the book ("Historical-Structural Dependency Analysis: Theory and Third World Development") reads like a PhD thesis with a pretty turgid style. For instance, few people will understand the sentence (p.19): "This multiplicity of local dependency structures implies a far

more variegated set of political options than the Manichean dichotomies brandished by Frank and Dos Santos". Basically Gereffi's thesis is that underdevelopment in the Third World is a consequence of capitalism and that nationalization of foreign-dominated industries — in this case the pharmaceutical industry — is the answer. The dependency theory is then tested in the much more readable second part ("A Crucial-case Test of Dependency Theory: The Steroid Hormone Industry in Mexico"), which is supposed to tell us something about the more general problem of "The Pharmaceutical Industry in a Third World Context", covered in the third part of the book.

The drug requirements of the Third World (LDCs) differ tremendously from those of technologically developed countries (DCs), which are the financial and managerial domiciles of the trans-national pharmaceutical companies. The DCs are basically geriatric countries with emphasis on costly geriatric medicine (cardiovascular diseases, cancer, expensive surgery and so on). In contrast, the LDCs are paediatric countries which, in addition to the usual problem of high infant mortality, are horribly burdened by parasitic diseases to which the DCs and, *ipso facto* the trans-

national pharmaceutical companies, pay little attention. The really important drugs for most LDC populations can be counted on the fingers of two hands, and with the possible exception of oral contraceptives they are not steroids. While the drug problems of the Third World are very real ones, the trans-national companies are neither going to provide the answer to them nor are they the key or only culprits. Economists almost always focus on the cost of drugs and their high profitability. While such considerations are not unimportant, the crucial factors are the national health care system that selects and distributes the drugs, and — even more fundamentally — the unmet drug requirements of the LDCs which are not a high priority of the international research and development constituency. The People's Republic of China and India are exceptions, in that they are not only self-sufficient in the manufacture of the key, basic drugs, but also do research on locally important health problems. The fairly recent programme of the World Health Organization on research in tropical diseases is an important exception where the priorities of the LDCs are taken into consideration and where trans-national companies play a relatively minor role.

These, however, are all topics which are peripheral for Gereffi. Instead he focuses on the remarkable story of the Mexican steroid industry. Steroids are glamour drugs — sex hormones, corticosteroids, diuretics, oral contraceptives — but except for contraceptives they really do not affect the poor, and the overall dollar volumes (well discussed by Gereffi) are all small compared to antibiotics, tranquillizers, and cardiovascular and anti-ulcer drugs.

During the 1940s, steroid drugs were expensive, were manufactured by a European cartel of pharmaceutical companies, and were limited to sex hormones and the adrenal hormone deoxycorticosterone. Most of them were produced by highly complicated and inefficient processes from cholesterol. In the late 1930s, Russell Marker, an American maverick chemist without a PhD (itself an amusing story), discovered that a steroidal sapogenin, diosgenin, is abundant in various tropical yams (the closest source to the United States being Mexico), and while working at Pennsylvania State College developed a highly efficient process which led directly to progesterone. According to Marker, American pharmaceutical companies were not interested and, in 1944, together with two Mexican entrepreneurs, he founded Syntex — a small pharmaceutical company. Gereffi tells this story well and it needs little embellishment by me. Suffice it to say, that, as a result of Syntex's entry, the high prices of the sex hormones produced in Europe dropped precipitously and basically led to the demise of the European hormone cartel with diosgenin becoming the preferred raw material. Around 1950,

Syntex's total sales were \$5 million or so and consisted exclusively of bulk export sales of standard hormones. Two events occurred at that time which changed the situation dramatically.

One was the enlightened decision by the owners of Syntex to establish a research laboratory and to bring to Mexico well-trained steroid chemists from North America and Europe, since the number of Mexican PhD organic chemists was tiny. The second event was the discovery of the therapeutic effects of cortisone — in 1950 a rare and very expensive drug — and the likelihood that corticosteroids used in arthritis might become the first steroid drug with sales in the many tens of millions of dollars. Syntex contributed heavily to that scientific field; apart from the work in its own laboratories, the entire research budget of the Institute of Chemistry of the National Autonomous University of Mexico was funded by the company and within a decade this led to a flourishing graduate programme in steroid and natural products chemistry. The impact of the imported European and American chemists (most of them directly or indirectly refugees from Hitler's Europe) upon the Mexican chemical scene is not mentioned at all by Gereffi; in my opinion it represented a major, albeit intangible, benefit which neither the Mexican politicians nor the economists ever recognized or calculated. The research programme was then extended from the development of new synthetic approaches to known steroids (sex hormones, cortisone) to the synthesis and development of new proprietary drugs. By the late 1950s, Syntex chemists had discovered the first oral contraceptive, the most powerful new topical corticosteroid, a novel anabolic agent and several other proprietary steroid drugs — a record of productivity virtually unequalled by any of the large trans-national companies with sales volumes of ten to several hundred times that of Syntex.

Syntex was well on the way to becoming the first trans-national pharmaceutical company founded and operating in a Third World country. In fact it is such a company now, its 1960 sales of less than \$10 million having multiplied by a factor of one hundred within a quarter of a century. However, neither the corporate headquarters nor most of the research facilities are now in Mexico. What went wrong? The factual account is well reported by Gereffi, but his conclusions are at best naive. Industrial corporations — large or small, trans-national or national (frequently the

most venial of them all), capitalist or socialist — are not philanthropic organizations and it is unreasonable to expect them to be more idealistic than the politicians running their respective countries. A good part of what Gereffi has missed can be found in my paper (*Proc. R. Soc. Lond. B.* 195, 175–188; 1976) which is absent from his otherwise extensive bibliography. This paper deals precisely with the question raised by Gereffi's Mexican example — “technical versus political aspects”. The last two sentences of the abstract tell it all:

The rise and decline of the steroid industry in Mexico is a sad but instructive example of how lack of resolution in the conflicting priorities (economic versus political) of multinational pharmaceutical companies and a developing country can lead to the possible dismantlement of a flourishing high technology. There are important differences between petroleum and steroids, which make the OPEC strategy non-viable in the steroid field.

The ultimately fatal mistake of the Mexicans was to concentrate on the price of a commodity — the yam. By doing so, they essentially made the choice in favour of commodities (yam, diosgenin, low “me-too” technology intermediates) and against research-orientated aims, which, for the first time in a Third World country, would have created a local pharmaceutical company of international calibre. In this context Gereffi makes some oversimplified and even incorrect generalizations — he cites Argentina as a country that actually managed to perform research leading to drug development by local companies. If one internationally recognized drug has come out of that research, it has escaped my attention. Gereffi talks about consumer welfare (p.118) and the fact that local government-sponsored companies were able to outbid Syntex on price concessions for drugs for the Mexican internal market (which in any event was piddling except for oral contraceptives). He forgets that these were “me-too” enterprises with no research and development behind them. Gereffi also claims (p.128) that corruption was part of “business as usual” in the Mexican steroid industry without making it clear that such corruption permeated the entire fibre of all Mexican business — indeed of Mexican life; it would have been impossible to operate in the 1945–1975 period without recourse to bribes.

The height of naivety, though understandable, was the demand in Mexico for nationalization of the steroid industry which led to Syntex's move from the country (pp.142–144). This was an OPEC-type strategy at its most inappropriate. Oil was a world-wide necessity, and nationalization coupled with ruthless raising of the price was feasible because there were no immediate alternatives. The 1970s, Mexico and yam-derived diosgenin simply did not fit such a picture. The moment the trans-national companies (by then Syntex had joined the club) realized what was going to happen, they took alternative steps —

notably, they looked at total synthesis and novel microbiological transformations. These steroid equivalents to the introduction of nuclear energy in lieu of oil took only a few years — not decades — and once the investment and manufacturing decision had been made, no lowering of the price of the Mexican commodity was ever going to reverse that decision to manufacture steroids elsewhere by a process which is not subject to sudden political moves.

One category of steroid drug — oral contraceptives — merits separate discussion. This is the only type of steroid that I would consider to be an essential component of the “bare-foot doctor's bag” of Third World countries. But even here one can make a case that simple economic analyses miss the point and that nationalization is no panacea. At one time, most of the world's oral contraceptives were manufactured in Mexico — the country of their discovery. Yet by 1972, when the Mexican government was still indifferent to problems of over-population, the writing of the demise of the Mexican steroid industry was already on the wall. Total synthesis (from air, coal and water) rather than partial synthesis (from diosgenin and other plant-derived intermediates) became economically competitive and politically prudent. Gereffi implies (p.134) that this also happened in the United States, with total synthesis (at that time only of oral contraceptives) entering the stage in 1973. This is untrue. To my knowledge, neither in 1973 or now, are any oral contraceptives manufactured by total synthesis in the United States. In fact, the active ingredients are not manufactured at all in the United States, but rather come from Germany and France. To that extent, the United States appears to be as dependent on foreigners as the Mexicans or other Third World countries. I doubt, however, whether this is reflected in the price paid by the American or Mexican consumer or that American companies or the United States government lose much sleep over it.

More telling is the point that almost any Third World country can get oral contraceptives free, or certainly at a lower price than they could ever manufacture them domestically. An example is the Philippines — a country that received such quantities of American donated oral contraceptives that much of the supply ended up in storage beyond the five-year expiration deadline. It was the inefficient local delivery system and infrastructure which was at fault, not the price of the drug.

Trans-national companies are not always the villains, and Third World dependency upon the international pharmaceutical industry is a grey question. Too many economists, Gereffi among them, make it appear black or white. □

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Cancer series

The first volume to emerge from a new series of conferences on the cancer cell has been published by Cold Spring Harbor Laboratory. *The Transformed Phenotype* is edited by A.J. Levine, G.F. Vande Woude, W.C. Topp and J.D. Watson, and is the record of a meeting held in September 1983 (reported in *Nature* 305, 470; 1983). Price of the book, a large-format flexicover, is \$45 (US), \$54 (elsewhere).

Being an astronomer, being a woman

Owen Gingerich

Cecilia Payne-Gaposchkin: An Autobiography and other Recollections.

Edited by Katherine Haramundanis.

Cambridge University Press: 1984.

Pp. 275. £19.50, \$34.50.

CECILIA Helena Payne-Gaposchkin was the most eminent woman astronomer of all time. In 1925, while a graduate student at Harvard Observatory, she applied the newly discovered Saha equations to an analysis of the temperatures of stellar atmospheres. Her solution was so thorough that afterwards temperatures of stars essentially became a non-problem, and most astronomers came to believe that the main thrust of her thesis concerned the *composition* of stars. In fact, though the analysis of chemical abundances was originally only ancillary to her study, it turned out to be the most important result: she had demonstrated that the great majority of stars had quite similar chemical abundances and that the widely varying appearances of stellar spectra arose from physical rather than compositional differences.

The Dyer's Hand, which constitutes the main part of this book, is a reflective but all-too-terse autobiographical account written near the end of her life. She brought the richness of an earlier English education to her writing, her book being filled with splendid though often cryptic literary allusions. Indeed, we may well wonder how far to read Shakespeare beyond the title quotation:

My nature is subdued
To what it works in, like the dyer's hand;
Pity me, then, and wish I were renewed.

In the short Part III of her account, entitled "The Dyer's Hand Subdued", she tells of her marriage to Sergei Gaposchkin, a stateless Russian emigré and brilliant variable-star astronomer, and of her own change of career from spectroscopy to photometry.

Payne-Gaposchkin was a modest woman. She firmly believed that scientific discoveries would inevitably be made, if not by one researcher, then by another, and her autobiography is the poorer for this. She lived in an age of heroic changes in our perception of the astronomical universe, and she knew many colourful figures who, like herself, contributed to these advances. However, she always bent toward the side of caution, taking care to include nothing "actionable" in her story. There are, nonetheless, wonderful moments in the book. In describing her undergraduate education in the English Cambridge, she wrote (p. 117):

The stress was on observation. 'One

thoroughgoing experiment,' Rutherford thundered in one of his lectures, 'is worth all the theories in the world — even if those theories are those of a Bohr.' Years later, Eddington uttered the dictum that he would not believe an observation unless it was supported by a good theory. I was an astronomer by that time and knew him well. I told him that I was shocked by his pronouncement. He smiled gently. 'I thought it would be good for Rutherford,' he said.

This edition has been enriched by a trio of introductory essays. Caltech's Jesse Greenstein, who first became acquainted with Payne-Gaposchkin when he was a student at Harvard, fills in the scientific background of her accomplishments. Peggy Kidwell, a professional historian of science who is currently writing a longer biography of Payne-Gaposchkin, presents a carefully documented outside view, especially of Cecilia Payne's early years at Harvard. Here we find, in sharper relief than in the autobiography, some of the difficulties facing a brilliant woman who was competing in a man's world. Finally, Payne-Gaposchkin's daughter, Katharine Haramundanis (the editor and prime mover in the preparation of this volume), gives an engaging picture of life in the Gaposchkin household. Her story significantly strengthens the book, for it fills in precisely where her mother's account is the weakest.

Important as Payne-Gaposchkin's astronomical contributions were, the

interest in this narrative clearly lies in the fact that it was written by a scientist who had an uphill struggle on account of her sex. She tells of teaching and advising Harvard graduate students in a day when no woman could be listed in the catalogue, and she frankly expresses her jealousy of Harry Plaskett, a contemporary astrophysicist whose advancement came so much easier than her own. Eventually Payne-Gaposchkin became the first woman professor to be promoted from the ranks at Harvard, and simultaneously the first woman to become the chairman of a department there. Not until 1977, at the age of 76, did she win the Henry Norris Russell lectureship, the highest honour of the American Astronomical Society, where she bluntly told the audience "Ten, 20, 30, 40, 50 years ago I could have given you news. Today I can offer only a panorama".

Never a militant women's liberationist, she expressed her attitude in her advice to young women (p. 227):

Do not undertake a scientific career in quest of fame or money. There are easier and better ways to reach them. Undertake it only if nothing else will satisfy you; for nothing else is probably what you will receive. Your reward will be the widening of the horizon as you climb. And if you achieve that reward you will ask no other. □

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Part analysis

Richard Compton

Electroanalytical Chemistry.

By Basil H. Vassos and Galen W. Ewing.

Wiley: 1984. Pp. 255. £39.85, \$51.75.

IN their preface, Vassos and Ewing tell us that they set themselves the task of writing a book to include "those aspects of electrochemistry, both theoretical and practical, that a graduate student specializing in analytical chemistry should master". Where they succeed in maintaining this selectivity, the book meets the needs of its intended readership — it contains clear, lively and up-to-date accounts of polarography, linear sweep, pulse and ac voltammetries, stripping analysis and hydrodynamic electrodes. The principles of these methods are well explained without the reader being burdened with excessive mathematics. In particular the sections on instrumentation and practice are excellent.

However the above topics occupy just over half of the book. The rest consists of a whirlwind tour through much of the remainder of electrochemistry. Thus topics such as classical thermodynamic electrochemistry, double-layer theory and electrocapillarity, reference electrodes, ion-selective electrodes, diffusion phenomena

and electrode kinetics all appear in the early chapters, but are treated with such brevity that novices will be forced to turn elsewhere to be able to proceed while the initiated will find little that is new to them. In the last quarter of the book the authors have added discussion of the fractional calculus, semiconductor electrochemistry and coupled electrochemical-spectroscopic methods; again, inclusion of this material seems at variance with the needs of their intended audience.

Overall, the book has the appearance of a textbook on modern electrochemical methods rather than one exclusively devoted to electroanalysis, but I suspect that those seeking such a book will choose either the superbly thorough work by Bard and Faulkner (*Electrochemical Methods*; Wiley, 1980), or the more idiosyncratic style of Albery (*Electrode Kinetics*; Oxford University Press, 1975), to name two textbooks in the field. Nevertheless where the authors stick to their brief the book is very good indeed. It merits the attention of those teaching courses on electroanalytical chemistry, and of analytical chemists in general — although one imagines that a book described on the dust-jacket as "extremely practical", but which contains but 19 lines on microprocessors, will date rather rapidly! □

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Nucleic acids change

S. Venitt

Molecular Biology of Mutagens and Carcinogens.

By B. Singer and D. Grunberger.

Plenum: 1983. Pp.347. \$37.50, £28.88.

WITH the continuing excitement over oncogenes and their role in malignant transformation, the use of the term "molecular biology" in relation to the study of cancer has an almost sacerdotal ring about it. The high priests of molecular biology will therefore be disappointed when they browse through *Molecular Biology of Mutagens and Carcinogens*, for apart from a veiled reference to the discovery of a point mutation in a cell derived from human bladder carcinoma (presumably the G to T transversion in the oncogene of the T24/EJ cell line), and a short chapter on site-directed mutagenesis, Singer and Grunberger's book contains little in the way of molecular biology as the term is now generally understood.

What the book does contain is a succinct yet valuable series of chapters summarizing the results of several decades of fundamental research on the reaction of carcinogens and mutagens with nucleic acids. Most of the work described is firmly based on the older disciplines of chemistry and biochemistry. To this extent, therefore, the title is misleading.

From the outset, the authors (both leading investigators in this area of research) declare their allegiance to the somatic mutation theory of chemical carcinogenesis by stating in their introduction that the initiation stage of carcinogenesis is the term they use for the covalent binding of a reactive group to a nucleotide in an informational macromolecule. They also equate "direct modification of nucleic acids" with "somatic mutation", a form of mental short-hand in which accuracy is the victim of brevity. There then follow chapters describing in detail the physical and chemical properties of nucleic acids and the great variety of ways in which very diverse kinds of chemicals (directly or following metabolism) can react with or modify the structure, function or repair of DNA. Description of recent techniques (of exquisite sensitivity) for the detection of DNA adducts such as post-labelling and radio-immune assay is included. Each chapter ends with a list of selected references, and a wealth of other references are included in legends to figures and tables. The book ends with about 70 very useful pages of UV spectra and dissociation constants of a wide range of naturally occurring and chemically modified bases, nucleosides and nucleotides.

There is some evidence of less than rigorous editing. For example, on page 3, the natural plant products saffrole and estragole are mentioned, as it were, in the

same breath as aflatoxin, being described as "some of the most potent carcinogens known". However, saffrole and estragole are in fact very weak carcinogens (as is made clear later in the text), being several orders of magnitude less potent than aflatoxin B₁.

Although this volume cannot compete either in scope or in depth with the monumental American Chemical Society Monograph 173, *Chemical Carcinogens* edited by C.E. Searle (of which a second edition is in press), it is nevertheless a helpful and up-to-date source of reference. Anyone interested in chemical mutagenesis and carcinogenesis, from undergraduate level upwards, will find it rewarding. □

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Beyond vision

T.W. Goodwin

The Retinoids, in two volumes.

Edited by Michael B. Sporn, Anita B. Roberts and DeWitt S. Goodman.

Academic: 1984. Vol. 1, pp.424, \$46, £35.50; Vol. 2, pp.446, \$48, £37.

It is some 70 years since "fat-soluble A" was shown to have growth-promoting activity in rats. This material was eventually purified to yield vitamin A, the chemical structure of which was determined in 1930 by Karrer. The first specific biological function of vitamin A was demonstrated in 1934 by Wald — who showed a related compound, retinene, was the co-factor of the protein rhodopsin which is the photo-sensitive pigment in vision — but it was not until 1944 that retinene was characterized as vitamin A aldehyde by Morton.

Since then developments in the study of vitamin A have been numerous, and many investigators have widened their horizons and moved away from the classical nutritional approach. Indeed so many compounds, both natural and synthetic, with vitamin A activity of some kind, have been now examined that a new nomenclature has been devised; hence the title of these volumes. Retinoids are "those diterpenoids derived from a monocyclic parent compound containing five C-C double bonds and a functional group at the terminus of the acyclic portion". Thus vitamin A is retinol and retinene is retinal. The term retinoid tends to emphasize that the only role of "vitamin A" fully explained at the biochemical level is its function in vision.

The two volumes are a collection of 16 authoritative essays on most aspects of retinoid chemistry, biochemistry, molecular biology and pharmacology. Clinical applications are also covered. All the contributors but one are from the United

States, the exception being F. Frinckel who has provided a comprehensive review on the chemistry and physical properties of the retinoids. There follow equally stimulating contributions on the production of labelled retinoids (by the late H.H. Kaegi), on extraction and separation with particular emphasis on the use of HPLC, and on biological assays, this last chapter including a valuable table which lists the activity of some 30 retinoids in nine assays. In spite of many efforts by enlightened workers, vitamin A deficiency in the Third World is still a grave nutritional problem and the topic deserves the thoughtful consideration provided here by Barbara A. Underwood. All these chapters are in the first volume which ends with an index and a 20-page appendix of structural formulae and specific names of known retinoids.

The publishers have generously repeated the appendix in the second volume, which is concerned with the metabolism, biological functions and clinical activity of retinoids. Retinol-binding protein (RBP) and cellular retinol-binding protein (CRBP) are discussed in detail, as is the role of the retinoids in photosynthetic systems. In contrast, the bias of the book towards animal function has inevitably led to a somewhat perfunctory coverage of the important bacteriorhodopsins.

For the rest of Vol. 2 we have summaries of new evidence for novel retinoid functions, none of which has yet been explained at the biochemical level. The most obvious function which should be amenable to biochemical explanation — glycosyl transfer reactions reflecting the changes in cell-surface glycoconjugates in growth and differentiation — still requires the clear demonstration of a role for retinoids distinct from dolichol and the mode of participation of retinoid acid, which is far more active than retinol in this system. This unsatisfactory state of affairs is thought by Roberts and Sporn to be due to the failure of investigators to consider in detail the central part of genes in these effects; indeed, we eagerly await a breakthrough indicating how retinoids control gene expression.

A controversial issue at the moment is the possible role of retinoids in cancer control. The review by Moon and Itri makes it clear that retinoids can modify differentiation of preneoplastic and neoplastic tissues, but the clinical potential requires much further assessment. However, one success story is the extremely effective action of these compounds in certain dermatological conditions.

Between them the two volumes provide a thorough-going, up-to-date and well-poised account of the current status of retinoid research. As such a wide variety of research workers and clinicians will need access to them. □

T.W. Goodwin is Emeritus Professor of Biochemistry at the University of Liverpool. Volume II (Animals) of his work The Biochemistry of the Carotenoids has recently appeared in a second edition.

Further problems of entertainment

David Singmaster

Wheels, Life and Other Mathematical Amusements.

By Martin Gardner.

W. H. Freeman: 1984. Pp.261.

\$15.95, £15.95.

MARTIN Gardner's fans, many of whom have been suffering withdrawal symptoms since his retirement from *Scientific American*, can take some comfort in the fact that the books of his collected columns are several years behindhand. *Wheels, Life and Other Mathematical Amusements* is his first collection since 1979 and covers the years 1970–1972.

Gardner's columns invariably attracted many other people to work on the problems he discussed and their results would be reported in later columns. In previous collections, such material was generally simply appended to the original article. However, the great lapse of time from the initial appearance of these articles has required much revision and addition to many of them, sometimes as long as the originals. Indeed, there is a whole new chapter on the Game of Life. Thus there is more new material in this collection than one would have expected.

Among the highlights is the account of non-transitive dice. One makes a set of four peculiarly numbered dice called A, B, C, D. These are used in a game where two players throw a die and the die with the larger number wins. Then die A is better than B, which is better than C, which is better than D, which is better than A! If you generously allow your opponent to pick his die first, and you pick the preceding die in the cycle, then you have a 2/3 chance of winning.

Rather different is "A Set of Quickies". Here Gardner has included one of his own problems: what is the largest number of unit cubes which can touch a unit cube properly (that is, with some portion of common face)? Gardner gave a solution with 20 cubes and was surprised to get solutions with 22, 23 and 24 cubes.

Whether 24 is maximal remains an open question. "Advertising Premiums" covers a variety of classical puzzles — especially those of Sam Loyd, who invented the Trick Donkeys, the Pony Puzzle, the Pencil on a Loop of String and Get off the Earth — while another chapter includes Charles Addams's Skier. The Addams cartoon shows a skier whose two tracks pass on either side of a tree. If you found such ski tracks, could you give at least six possible explanations?

Finally, three chapters deal with John Conway's Game of Life, which was first published by Gardner and promptly consumed many hours of computer time, even generating a newsletter which ran for several years. A further chapter contains new material and brings the story up to date.

If you know Gardner's works, you will need no encouragement to buy this book; if you don't, you will find it a delightful introduction to the most popular mathematical writer of our time. □

David Singmaster is Reader in Mathematics at the Polytechnic of the South Bank, London.

Chemist's wood

Gavin S. Hall

Wood: Chemistry, Ultrastructure, Reactions.

By D. Fengel and G. Wegener.

Walter de Gruyter: 1984. Pp.613. DM 245.

Wood, the main title (and indeed the only title on the spine) does not give much of a clue as to the subject matter and scope of this substantial book. The sub-title, however, provides an accurate pointer to the contents which deal primarily with the chemical characteristics of wood — the ligno-cellulosic raw material of which trees and shrubs are composed. As would be expected with such an important resource, the subject is already well documented and there are a number of books on the subject. The authors acknowledge this fact and state as their aim the presentation of a comprehensive account of progress in wood chemistry based on information published between 1960 and 1982. Their book

therefore builds on earlier, standard works such as *Wood Chemistry* by L. E. Wise and E. C. Jahn (Reinhold, 1952), *The Chemistry of Lignin* by F. E. Brauns and D. A. Brauns (Academic, 1960) and *The Chemistry of Wood* by B. L. Browning (Interscience, 1963).

Of the 18 chapters, averaging some 30 pages each, six are devoted to chemical composition, constituents and ultrastructural relationships. One of these deals with the often neglected but increasingly significant component of forest crops — bark, and gives a concise account of its general anatomy and chemical composition. Two further chapters deal with the structure and ultrastructure of wood from the chemist's viewpoint. The second half of the book concerns itself with the reactions of wood in relation to environmental factors, such as light, heat and micro-organisms, acids and alkalis, and then with industrial processing for pulp and chemical derivatives.

One of the notable features is the list of references found at the end of each chapter. Some idea of the thoroughness with which the authors have undertaken their task can be gauged by the fact that in all there are some 2,900 citations (or 160 per chapter). If trying hard to find fault, one might have asked for more obvious critical analysis and interpretation of the source material. However, overall this is a very competent, readable review of the literature, one produced by acknowledged specialists in the field. The style and form of presentation are such that newcomers will be stimulated to delve deeper into the various subject areas, a process greatly facilitated by the references to literature surveyed. The linguistic versatility of the authors has clearly enhanced the value of this aspect of the book.

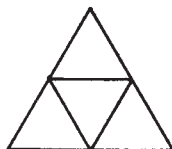
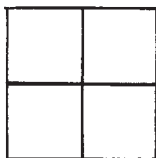
Wood is well-produced, with an appropriate leavening of tabular, diagrammatic and photographic material. It will serve well either as a refresher course for those whose interest or occupations involve wood chemistry, or as a starting point for the student seeking to specialize in the subject. □

Gavin S. Hall is Head of Technology at the Timber Research and Development Association, High Wycombe, Buckinghamshire.

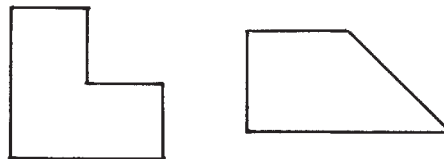
Divisive difficulties from David Singmaster

The following problems start from an old chestnut, but have a new twist. The solutions will appear in *Nature* of 23 August.

It is easy to divide a square or an equilateral triangle into four congruent areas as shown below.



A. Divide these two figures into four congruent areas.



B. When you have done Part A, try to divide a square into five congruent areas.

C. If you find Part B easy or you already know the solution, then find another solution which is not symmetric to the first solution. (Hint: many people will consider the other solutions unacceptable! It is not known if any "acceptable" solutions exist.)

Mathematics

Approximation of Nonlinear Evolution Systems. Mathematics in Science and Engineering, Vol. 164. By JOSEPH W. JEROME. Academic: 1983. Pp. 280. ISBN 0-12-384680-3. \$46.

Generalized Functions: Theory and Technique. Mathematics in Science and Engineering, Vol. 171. By RAM P. KANWAL. Academic: 1983. Pp. 427. ISBN 0-12-396560-8. \$58.

Structural Equation Geometry: The Inherent Properties of Curves and Coordinate Systems. By J. LEE KAVANAU. Science Software Systems: 1983. Pp. 506. Pbk ISBN 0-937292-02-8. Pbk \$16.95.

Cosmology and Space Science

Amateur Astronomy: A Comprehensive and Practical Survey. COLIN RONAN (ed.). Newnes Books: 1984. Pp. 256. ISBN 0-600-35667-1. £8.95.

Amateur Astronomy Pocket Skyguide. By MARK R. CHARTRAND III. Newnes Books: 1984. Pp. 272. Pbk ISBN 0-600-35708-2. Pbk £3.95.

Galactic and Extragalactic Infrared Spectroscopy. M.F. KESLER and J.P. PHILLIPS (eds). Reidel: 1983. Pp. 472. ISBN 90-277-1704-4. Dfl. 155, \$58.

The Illustrated Encyclopedia of Space Exploration. By RICHARD S. LEWIS. Salamander Books: 1984. Pp. 320. ISBN 0-86101-181-3. £12.95.

In Search of Ancient Astronomies. E.C. KRUPP (ed.). Penguin: 1984. Pp. 281. ISBN 0-14-00-5481-2. Pbk £4.95.

The Star Book. By ROBERT BURNHAM. Cambridge University Press: 1984. Pp. 17. Pbk ISBN 0-521-25833-2. Pbk £3.50.

Physics

Advances in Infrared and Raman Spectroscopy. Vol. 11. R.J.H. CLARK and R.E. HESTER (eds). Wiley: 1983. ISBN 0-471-26267-6. £49.50.

Astronomy and Astrophysics Abstracts. Vol. 33. S. BÖHME et al. (eds). Springer-Verlag: 1983. Pp. 815. ISBN 3-540-13017-9/0-387-13017-9. DM 118.40, \$46.

Electron-Hole Droplets in Semiconductors. C.D. JEFFRIES and L.V. KELDYSH (eds). North-Holland: 1983. Pp. 656. ISBN 0-444-86530-6. Np.

Essays in Theoretical Physics: In Honour of Dirk ter Haar. W.E. PARRY (ed.). Pergamon: 1984. Pp. 337. ISBN 0-08-026523-5. £28.00, \$45.00.

Gravi-Magnetism. By J. BIEMOND. Sansovinostraat 28, 5624JX Eindhoven, The Netherlands: 1984. Pp. 22. Pbk ISBN 90-9000621-4. \$4, Dfl. 10.

The History of Physics. By ISAAC ASIMOV. Walker: 1984. Pp. 762. ISBN 0-8027-8027-0751-3. \$29.95.

Introduction to Supersymmetry in Particle and Nuclear Physics. O. CASTANOS, A. FRANK and L. URRUTIA (eds). Plenum: 1984. Pp. 187. ISBN 0-306-41612-3. Np.

Technology

Chemical Reactor Design and Operation. By K.R. WESTERTERP, W.P.M. VAN SWAAIJ and A.A.C.M. BEENACKERS. Wiley: 1984. Pp. 767. ISBN 0-471-90183-0. £49.50.

The Future for Automotive Technology. by ULRICH SEIFFERT and PETER WALZER. Frances Pinter: 1984. Pp. 197. ISBN 0-86187-460-9. £15.

The Future for Space Technology. By GEOFFREY K.C. PARDOE. Frances Pinter: 1984. Pp. 206. ISBN 0-86187-462-5. £14.95.

The Science and Technology of Coal and Coal Utilization. BERNARD R. COOPER and WILLIAM A. ELLINGSON (eds). Plenum: 1984. Pp. 666. ISBN 0-306-41436-8. \$85.

Wayward Technology. By ERNST BRAUN. Frances Pinter: 1984. Pp. 224. ISBN 0-86187-288-6. £15.50.

Zeolites: Science and Technology. F. RAMOA RIBEIRO et al. (eds). Kluwer: 1984. Pp. 698. ISBN 90-247-2935-1. \$85, £57.25.

Computer Science

Advances in Data Base Theory. Vol. 2. HERVE GALLAIRE, JACK MINKER and JEAN MARIE NICOLAS (eds). Plenum: 1984. Pp. 428. ISBN 0-306-41636-0. \$59.50.

Personal Computer Book, 3rd Edn. By ROBIN BRADBEER. Gower: 1984. Pp. 280. Hbk ISBN 0-566-03507-3; pbk ISBN 0-566-03506-5. Hbk £9.50, pbk £5.95.

The Software Catalog Science and Engineering. Produced from the International Software Database. Elsevier: 1984. Pp. 687. Pbk ISBN 0-444-00925-6. \$29.

Earth Sciences

Acoustic Waveguides: Applications to Oceanic Science. By C. ALLAN BOYLES. Wiley: 1984. Pp. 321. ISBN 0-471-88771-4. £44.60.

Advances in Petroleum Geochemistry. Vol. 1. JIM BROOKS and DIETRICH WELTE (eds). Academic: 1984. Pp. 344. ISBN 0-12-032001-0. £35, \$49.50.

Cloud Dynamics. By L.T. MATVEEV. Reidel: 1984. Pp. 340. ISBN 90-277-1737-0. Dfl. 190, \$71.

Large-Scale Dynamical Processes in the Atmosphere. B.J. HOSKINS and R.P. PEARCE (eds). Academic: 1983. Pp. 397. ISBN 0-123-56680-0. \$54.50.

Metal Deposits in Relation to Plate Tectonics. By F.J. SAWKINS. Springer-Verlag: 1984. Pp. 325. ISBN 3-540-12752-6/0-387-12752-6. DM 98, \$38.10.

Metamorphic Complexes of Asia. V.S. SOBOLEV (ed.). Pergamon: 1984. Pp. 324. ISBN 0-08-022854-2. £33.50, \$60.

The Morphostructure of the Atlantic Ocean Floor. BY V.M. LITVIN. Reidel: 1984. Pp. 172. ISBN 90-277-1509-2. Dfl. 95, \$36.50.

Ocean Tides: Mathematical Models & Numerical Experiments. By G.I. MARCHUK and B.A. KAGAN. Pergamon: 1984. Pp. 292. ISBN 0-08-026236-8. £36, \$65.

Ophiolites and Oceanic Lithosphere. Geological Society Special Publication No 13. I.G. GASS, S.J. LIPPARD and A.W. SHELTON (eds). Blackwell Scientific: 1984. Pp. 413. ISBN 0-632-01219-6. £37.50.

Principles of Lake Sedimentology. By L. HAKANSON and M. HANSSON. Springer-Verlag: 1983. Pp. 316. ISBN 3-540-12645-7/0-387-12645-7. DM 98, \$38.10.

Shorelines and Isostasy. D.E. SMITH and A.G. DAWSON. Academic: 1984. Pp. 387. ISBN 0-12-652960-4. \$55.

West Africa: Geological Introduction and Stratigraphic Terms. J. FABRE (ed.). Pergamon: 1983. Pp. 396. ISBN 0-08-030267-X. £36, \$65.

Environmental Sciences

The Handbook of Environmental Chemistry: Anthropogenic Compounds. O. HUTZINGER (ed.). Springer-Verlag: 1984. Pp. 220. ISBN 3-540-13019-5/0-387-12019-5. DM 138, \$51.50.

Health Effects of Low-Level Radiation. By WILLIAM R. HENDEE. Prentice-Hall: 1984. Pp. 266. ISBN 0-8385-3666-2. £23.70, \$33.70.

Heavy Metals in Natural Waters: Applied Monitoring and Impact Assessment. By JAMES W. MOORE and S. RAMAMOORTHY. Springer-Verlag: 1984. Pp. 268. ISBN 3-540-90885-4/0-387-90885-4. DM 108, \$40.30.

General

Analytical Calorimetry. Vol. 5. JULIAN F. JOHNSON and PHILIP S. GILL (eds). Plenum: 1984. Pp. 402. ISBN 0-306-41507-0. \$69.50.

Aquametry, 3rd Edn. Part II Electrical and Electronic Methods. By DONALD MILTON SMITH and JOHN MITCHELL Jr. Wiley: 1984. Pp. 1352. ISBN 0-471-02265-9. £156.75.

Aspects of Vagueness. Theory and Decision Library. Vol. 39. HEINZ J. SKALA et al. (eds). Reidel: 1984. Pp. 304. ISBN 90-277-1692-7. Dfl. 105, \$39.50.

Back Pain: The Facts. By MALCOLM I.V. JAYSON. Oxford University Press: 1984. Pp. 182. Pbk ISBN 0-19-286042-9. Pbk £2.95.

Bioscope, 2nd Edn. By THOMAS A. EASTON and CARL E. RISCER. Charles E. Merrill: 1984. Pp. 719. Pbk ISBN 0-675-20137-3. Np.

Bioscope, 2nd Edn. Student Guide. By CAROL A. WELSH. Charles E. Merrill: 1984. Pp. 266. Pbk ISBN 0-675-20233-7. Np.

The Changing Definition of Masculinity. By CLYDE W. FRANKLIN II. Plenum: 1984. Pp. 234. ISBN 0-306-41534-2. Np.

Classical Potential Theory and its Probabilistic Counterpart. By J.L. DOOB. Springer-Verlag: 1984. Pp. 846. ISBN 3-540-90881-1/0-387-90881-1. \$62.70.

Darwin's Legacy. Nobel Conference XVIII. CHARLES L. HAMRUM (ed.). Harper & Row: 1984. Pp. 125. Pbk ISBN 0-06-250651-X. Pbk £3.95.

East Anglian Landscapes: Past and Present. By JACK RAVENSDALE and RICHARD MUIR. Michael Joseph: 1984. Pp. 256. ISBN 0-7181-2343-3. £12.95.

E.C. Stakeman: Statesman of Science. By C.M. CHRISTENSEN. The American Phytopathological Society: 1984. Pp. 156. ISBN 0-89054-056-X. \$18.

Epilepsy: The Facts. By ANTHONY HOPKINS. Oxford University Press: 1984. Pp. 159. Pbk ISBN 0-19-286041-0. Pbk £2.95.

Great Rivers of the World. ALEXANDER FRATER (ed.). Hodder & Stoughton: 1984. Pp. 160. ISBN 0-340-27998-2. £12.95.

Handbook of U.S. Colorants for Foods, Drugs and Cosmetics, 2nd Edn. By DANIEL M. MARMION. Wiley: 1984. Pp. 466. ISBN 0-471-09312-2. Np.

Horse Management. JOHN HICKMAN (ed.). Academic: 1984. Pp. 339. ISBN 0-123-47220-2. Np.

Into Thin Air: A History of Aviation Medicine in the RAF. By T.M. GIBSON and M.H. HARRISON. Robert Hale: 1984. Pp. 279. ISBN 0-7090-1290-X. £12.50.

Just in Case: A Passenger's Guide to Airplane Safety and Survival. By DANIEL A. JOHNSON. Plenum: 1984. Pp. 268. ISBN 0-306-41576-3. Np.

Landscape Ecology: Theory and Application. By ZEY NAVEH and ARTHUR S. LIEVERMAN. Springer-Verlag: 1984. Pp. 356. ISBN 3-540-90849-8/0-387-90849-8. DM 108, \$40.30.

Liquid Crystals and Ordered Fluids. Vol. 4. ANSELM C. GRIFFIN and JULIAN F. JOHNSON (eds). Plenum: 1984. Pp. 1157. ISBN 0-306-41394-9. Np.

The Lost Australia of François Péron. By COLIN WALLACE. Nottingham Court Press: 1984. Pp. 164. ISBN 0-906691-96-6. £9.95.

Marine Biodeterioration: An Interdisciplinary Study. J.D. COSTLOW and R.C. TIPPER (eds). E. & F.N. Spon: 1984. Pp. 384. ISBN 0-419-13340-2. £25.

Multicritical Phenomena. R. PYNN and A. SKJELTORP (eds). Plenum: 1984. Pp. 472. ISBN 0-306-41625-5. Np.

Mysterious America. By LOREN COLEMAN. Faber & Faber: 1984. Pp. 301. Pbk ISBN 0-571-12524-7. Pbk £6.95.

Number Theory in Science and Communication with Applications in Cryptography, Physics, Biology, Digital Information, and Computing. By M.R. SCHROEDER. Springer-Verlag: 1984. Pp. 324. ISBN 3-540-12164-1/0-387-12164-1. £23.90.

Political Science Abstracts Index. 3 Vols. SAMUEL LONG (ed.). IFI/Plenum: 1983. Vol. 1 pp. 670, ISBN 0-306-69032-2; Vol. 2 pp. 1368, ISBN 0-306-69032-2; Vol. 3 pp. 2060, ISBN 0-306-69032-2. Np.

Quaternary Science Reviews. Vol. 1. D.Q. BOWEN (ed.). Pergamon: 1984. Pp. 319. ISBN 0-08-031491-0. £52.50, \$84.

Scientific Interference, Data Analysis, and Robustness. G.E.P. BOX et al. (eds). Academic: 1983. Pp. 304. ISBN 0-12-121160-6. \$22.50.

The Search for Certainty. By WILFORD W. SPRADLIN and PATRICIA PORTERFIELD. Springer-Verlag: 1984. Pp. 248. Pbk ISBN 3-540-90889-7/0-387-90889-7. Pbk \$36.60.

Sparse Matrix Technology. By SERGIO PISSANETSKY. Academic: 1984. Pp. 321. ISBN 0-12-557580-7. £32.50, \$55.

Spiritual Considerations in the Prevention, Treatment and Cure of Disease. By JANE H. THOMPSON. Oriel Press: 1984. Pp. 111. Hbk ISBN 0-85362-211-6; pbk ISBN 0-85362-212-4. Hbk £6.95; pbk £3.95.

Stochastic Phenomena and Chaotic Behaviour in Complex Systems. P. SCHUSTER (ed.). Springer-Verlag: 1984. Pp. 271. ISBN 3-540-13194-9/0-387-13194-9. \$31.70.

Termitologia: Fondation des Sociétés Construction. By PIERRE GRASSÉ. Masson: 1984. Pp. 613. ISBN 2-225-79959-8. Np.

Transport Processes in Wood. Springer Series in Wood Science. By JOHN F. SIAU. Springer-Verlag: 1984. Pp. 245. ISBN 3-504-12574-4/0-387-12574-4. DM 89, \$34.60.

Appointments

Dr J P Griffin has been appointed as the new Director of the Association of the British Pharmaceutical Industry, while **Mr D G Taylor**, formerly Deputy Director of the Office of Health Economics, has been appointed as the Association's first Director of Economic Planning.

Professor R L Bell, **Mr R Halstead**, **Professor Sir Hans Kornberg**, **Mr J S Martin**, **Mr J E Moffitt**, **Dr A M Raven** and **Sir Ralph Riley** have been appointed as members of the new Priorities Board for Research and Development, at the Ministry of Agriculture, Fisheries and Food.

Meetings

20-26 August, **Valcamonica Symposium-1984. Rock Art: New Horizons in Research**, Capo di Ponte, Italy (Symposium Secretary, Centro Cammuno di Studi Preistorici, 25004 Capo di Ponte, Brescia, Italy).

23-25 August, **International Symposium on Genetic Disorders: Medical Genetics, Past, Present and Future**, Oslo, Norway (Prof. Kåre Berg, Institute of Medical Genetics, University of Oslo, PO Box 1036, Blindern, 0315 Oslo 3, Norway).

27-30 August, **12th Symposium of the International Association for Hydraulic Research**, University of Stirling, UK (Mr Peter Collier, The National Engineering Laboratory, East Kilbride, Glasgow G75 0QU, UK).

28 August-5 September, **Advanced Course on: Oxygen and Sulfur Radicals in Chemistry and Medicine**, followed by **4th International Symposium on: Hypoxic Cell Radiosensitizing Drugs: The First and Second Generations on Cancer Treatment**, Bologna, Italy (Dr D. Tonelli, Istituto Scienze Chimiche, Università di Bologna, Via S. Donato 15, 40127 Bologna, Italy).

31 August-5 September, **4th World Congress on Pain**, Seattle, USA (Mr and Mrs Eugene H. Kone, 4th World Congress on Pain, 280 Knollwood Drive, New Haven, Connecticut 06515 USA).

3-7 September, **3rd International Congress of Inflammation**, Paris, France (F. Russo-Marie, Institut Pasteur, 28, Rue du Dr Roux, 75015 Paris, France).

4-6 September, **4th European Conference and Exhibition on Coal Utilization**, Essen, FRG (Industrial Presentations (Europe) B.V.'s Gravelandseweg 284-296, 3125 BK Schiedam, the Netherlands).

4-6 September, **Analyticon 84, the Second Conference for Analytical Science**, London, UK (Mr G.C. Young, Scientific Instrument Manufacturers' Association of Great Britain, Leicester House, 8 Leicester Street, London WC2H 7BN, UK).

4-7 September, **1st Annual Convention of the Institution of Diagnostic Engineers**, London, UK (Institute of Diagnostic Engineers, 3 Wycliffe Street, Leicester LE1

5LR, UK).

5-7 September, **9th Annual Symposium, Uranium Institute**, London, UK (Ms R.M. Stanger, Symposium Secretariat, Conference Associates UIS, 34 Stanford Road, London W8 5PZ, UK).

9-15 September, **Workshop on Experimental Insect Neurobiology**, Friday Harbor, USA (John Palka, Organizing Committee, Department of Zoology, NJ-15, University of Washington, Seattle, Washington 98195, USA).

10-13 September, **International Conference on Dielectric Materials, Measurements and Applications**, University of Lancaster, UK (The Institution of Electrical Engineers, Savoy Place, London WC2R 0BL, UK).

10-13 September, **8th International Symposium on Chemical Reaction Engineering** Edinburgh, UK (Ms Julie Wearne, The Conference Section, The Institution of Chemical Engineers, 165-171 Railway Terrace, Rugby, CV21 3HQ, UK).

10-14 September, **3rd European Congress on Biotechnology**, Munich, FRG (Congress Secretariat, DECHEMA, 3ECB, POB 970146, D-6000, Frankfurt am Main 97, FRG).

12-14 September, **Association of British Climatologists' Autumn Meeting on Coastal and Upland Climates**, University of Stirling, UK (Mr D.G. Tout, Department of Geography, University of Manchester, Manchester M13 9PL, UK).

14-16 September, **Conference on the Geology and Genesis of Mineral Deposits in Ireland**, Trinity College, Dublin, Ireland (Mr John Ashton, Secretary, the Irish Association for Economic Geology, Tara Mines Geology Department, Knockumber, Navan, Co. Meath, Ireland).

16-20 September, **International Symposium on Plasticity and Development of the Mammalian Spinal Chord**, Spoleto, Italy (Dr Alfredo Gorio, Fidia Research Laboratories, PO Box 110, 35051 Abano Terme, Italy).

17-19 September, **International Conference on Tribology in Mineral Extraction**, Nottingham, UK (Conference Officer, the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1H 9JJ, UK).

17-19 September, **Symposium on Humane Control of Land Mammals and Birds**, University of Surrey, UK (University Federation for Animal Welfare, 8 Hamilton Close, South Mimms, Potters Bar, Herts EN6 3QD, UK).

17-21 September, **5th Philips Conference on X-Ray Diffraction**, University of Exeter, UK (Philips Analytical Department, Pye Unicam Ltd, York Street, Cambridge CB1 2PX, UK).

17-21 September, **12th Biennial Conference of the International Association on Water Pollution Research and Control (IAWPRC)**, Amsterdam (IAWPRC, 29/30 High Holborn, London WC1V 6BA, UK).

18-19 September, **National Symposium on Exploiting the Laser in Engineering Production**, Coventry, UK (John Sanders, Manager, Communications, The Welding Institute, Abington Hall, Abington, Cambridge CB1 6AL, UK).

18-20 September, **The Royal Society of Chemistry's Autumn Meeting**, University of Hull, UK (Dr John F. Gibson, Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

18-20 September, **Oil Industry Nurses Symposium**, University of Oxford, UK (Ms C.H. Little, Conference Officer, Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR, UK).

24-25 September, **Seminar of Enhanced Biological Phosphorous Removal from Wastewater**, Paris, France (Dr M. Florentz, Phosphorus Seminar, Anjou-Recherche, 52 Rue d'Anjou, 75384 Paris Cedex 08, France; organised by the International Association on Water Pollution Research and Control).

26 September, **Symposium on Developments in Testing Concrete for Durability**, London, UK (Ms Anthea Wright, The Concrete Society, Devon House, 12-15 Dartmouth Street, London SW1H 9BL, UK).

26 September, **Seminar on Automated Assembling - A Step by Step Approach**, London, UK (Manager, Conferences and Exhibitions; The Institution of Production Engineers, Rochester House, 66 Little Ealing Lane, London W5 4XX, UK).

1-3 October, **10th European Colloquium on Heterocyclic Chemistry**, Kaiserslautern, FRG (Prof. Dr Manfred Regitz, Universität Kaiserslautern, Fachbereich Chemie, Erwin-Schrödinger-Strasse, D-6750 Kaiserslautern, FRG).

1-4 October, **51st Clean Air Conference**, Brighton, UK (National Society for Clean Air, 136 North Street, Brighton BN1 1RG, UK).

2-4 October, **International Symposium: Calcitonin 84**, Milan, Italy (Dr Maria Luisa Pecile, Department of Pharmacology, Chemotherapy and Medial Toxicology, University of Milan, 32 Via Vanvitelli, 20129 Milan, Italy).

3-5 October, **International Meeting on: Risk Assessment of Occupational Exposures in the Harbour Environment**, Genova, Italy (Dr Marina Vercelli, Scientific Secretary, 1st Oncologia Univ. Genova, Viale Benedetto XV, 10-16132 Genova, Italy).

3-5 October, **1984 Arctic Science Conference on Science and Public Policy**, Anchorage, USA (Department of Conferences and Institutes, University of Alaska - Fairbanks, 117 Eielson Building, Fairbanks, Alaska 99701).

5-6 October, **First Congress of the European Society of Nuclear Magnetic Resonance in Medicine**, Geneva, Switzerland (Mr Guy Delaborde, 18 Rue des Moraines, CH-1227 Geneva/Carouge, Switzerland).

ANNOUNCEMENTS

8-9 October, **Annual Bristol-Myers Cancer Research Symposium on Genetics, Cell Differentiation and Cancer**, New York (Suzanne C. Emery, Coordinator, Seventh Annual Bristol-Myers Symposium, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA).

8-12 October, **International Conference on Health Hazards and Biological Effects of Welding Fumes and Gases**, Copenhagen (Director, Environmental Health Service, WHO, Regional Office for Europe, 8 Scherfigsvej, 2100 Copenhagen, Denmark).

9-12 October, **16th Annual Meeting of the Division for Planetary Sciences of the American Astronomical Society**, Hawaii (Mr T.B. McCord, Program Chairman, Planetary Geosciences Division, Hawaii Institute of Geophysics, University of Hawaii, 2525 Correa Road, Honolulu, Hawaii 96822).

16 October, **Meeting on Management of External and Internal Overexposure** organised by the Society for Radiological Protection, London (Professor J.H. Martin, Programme Committee Secretary, Department of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee DD1 4HN, UK).

16-17 October, **3rd International Conference of the Institute of Energy on Fluidised Combustion**, London (Conference Department, Institute of Energy, 18 Devonshire Street, London W1N 2AU, UK).

17-18 October, **Meeting on Technology in the 1990s: Agriculture and Food**, London (Miss C.A. Johnson, the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

18-19 October, **1st European Seminar on Computer-Aided Molecular Design**, London (Ms. Helen Raquet, Oyez Scientific and Technical Services Ltd., Bath House (3rd floor), 56 Holborn Viaduct, London EC1, UK).

23 October, **Meeting on Air Filtration in the Nuclear Industry**, Manchester, UK (Hon. Secretary, Filtration Society, Department of Chemical Engineering, University of Technology, Loughborough, Leicestershire, UK).

23-24 October, **Meeting on Additives, Adjuvants and Preservatives in the Improvement of Food Quality** (in french), Lyon, France (Ms Christiane Rochas, Institut Pasteur de Lyon, 77, Rue Pasteur, 69365 Lyon Cedex 2, France).

28-30 October, **15th Meeting of the Underwater Mining Institute**, Madison, USA (Mr J.R. Moore, Program Chairman, University of Texas at Austin, Marine Science Institute, 200 East 261/2 Street, Austin, Texas 78705, USA).

28-29 October, **International Conference on Synthetic Antigens**, Siena, Italy (Prof. Paolo Neri, Conference Scientific Secretary, "Sclavo S.p.A.", via Fiorentina 1, 53100 Siena, Italy).

29-31 October, **Symposium on Lunar**

Bases and Space Activities of the 21st Century, Washington DC, USA (Mr Michael B. Duke, Chairman, Program Committee, NASA, Lyndon B. Johnson Space Center, Houston, Texas 77058, USA).

31 October-3 November, **35th Annual Meeting of the American Society of Human Genetics**, Toronto, Canada (Mr Gerry Gurvitch, Executive Secretary, Executive Office, PO Box 6015, Rockville, Maryland 20850, USA).

31-7 November, **Regional Assembly of the International Association of Seismology and Physics of the Earth's Interior**, Hyderabad, India (Dr. Mohan L. Gupta, Secretary Organizing Committee, IASPEI Regional Assembly, National Geophysical Research Institute, Hyderabad-500 007, India).

5-7 November, **Seminar on New Approaches to Rural Development and Conservation**, Castleton, UK (Mr Peter Townsend, Principal, Peak National Park Study Centre, Losehill Hall, Castleton, Derbyshire S30 2WB, UK).

9-11 November, **6th Congress of the Association of Radiation Oncologists of India**, Hubli, India (Dr A C Deka, Organising Secretary, Karnatak Cancer Therapy and Research Institute, Navangan, Hubli 580 025, Karnatak, India).

11-15 November, **Meeting on Filtration in the Automotive and Metalworking Industries**, Flint, Michigan, USA (Hon. Secretary, Filtration Society, Department of Chemical Engineering, Loughborough, Leicestershire, UK).

14 November, **Review Seminar on Extinctions: Their Rate and Causes Through Geological Time**, Milton Keynes, UK (Dr. P.W. Skelton, Department of Earth Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK).

16 November, **Meeting on Hyperthermia**, London, UK (Ms Janet Husband, The British Institute of Radiology, 36 Portland Place, London W1N 3DG, UK).

18-20 November, **7th Conference on Medical Physics of the Association of Medical Physicists of India**, Calcutta, India (Mr A Bose, Organising Secretary, Department of Radiotherapy, Cancer and Welfare Home, Mahatma Gandhi Road, Thakurpur, Calcutta 700 063, India).

19-22 November, **The 1984 Conference of the British Crop Protection Council**, Brighton, UK (Mr Frank Bishop, Conference Planners, 2A Kidderminster Road, Croydon, Surrey CRO 2UE, UK).

21-22 November, **Conference on Offshore and Onshore Engineering Practices Compared**, Glasgow, UK; and 27-29 November, **Extraction '84**, Dounreay, UK (The Conference Section, The Institute of Chemical Engineers, 165-171 Railway Terrace, Rugby CV21 3HQ, UK).

6 December, **The Meeting on Hormones and Breast Cancer**, Nice, France (Société Française de Cancérologie - ex Association Française pour l'Etude du Cancer - 26 Rue d'Ulm, 75231 Paris Cédex 05, France).

11-13 December, **16th Annual Meeting of The British Medical Ultrasound Society**, Harrogate, UK (Dr S Haskey, X-Ray Department, Harrogate District Hospital, Lancaster Park Road, Harrogate HG2 7SX, UK).

The Imperial Cancer Research Fund, is holding a series of seminars; on 1 August **Biological Functions of Calmodulin**; on 27th September, **Clonogenic Biology of Human Malignant Melanoma**; on 17 October, **Protein Phosphorylation and Hormone Action - an Overview**; on 21 November, **Salutory Replication and the Generation of Amplified Genes** (Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2).

During 1985, the University of California, at Los Angeles (UCLA) is again holding its **Annual Symposia series - on Molecular and Cellular Biology**: 19-26 January, **Nuclear Envelope Structure and RNA Maturation** (Steamboat Springs, Colorado); 26 January-2 February, **Monoclonal Antibodies and Cancer Therapy** (Park City, Utah); 27 January-2 February, **Leukemia 1985** (Keystone, Colorado); 2-8 February, **Perspectives in Inflammation, Neoplasia and Vascular Cell Biology** (Park City, Utah); 9-15 March, **Membrane Skeletons and Cytoskeletal-Membrane Associations** (Park City, Utah); 15-22 March, **Molecular Biology of Muscle Development** (Park City, Utah); 30 March-6 April, **Sequence Specificity in Transcription and Translation** (Steamboat Springs, Colorado); 30 March-4 April, **Molecular Determinants of Animal Form** (Park City, Utah); 6-12 April, **Biochemical and Molecular Epidemiology of Cancer** (Steamboat Springs, Colorado); 9-15 April, **Yeast Cell Biology** (Keystone, Colorado); 13-19 April, **Molecular Genetics of Filamentous Fungi** (Keystone, Colorado); 13-19 April, **Plant Genetics** (Keystone, Colorado); 20-25 April, **Options for the Control of Influenza** (Keystone, Colorado). For further details write to Ms Betty Handy, Program Coordinator, UCLA Symposia, Molecular Biology Institute, University of California, Los Angeles, CA 90024, USA.

Miscellaneous

The British Laboratory Ware Association is organizing a joint venture to visit **CHINACHEM**, a major exhibition for the Chemical and Petrochemical industries, in Tianjin, China, from 12-17 November 1984. Further information from Ms Karina Bateman at the BLWA, 30/32 Worple Road, London SW19 4EF, UK.

The Department of Chemistry at Lancaster University is launching a new Master of Science postgraduate course this October, on **New Polymer Synthesis**. The course runs for 12 months and further details are available from Mr Roger Grinyer, Information Officer, University House, Bailrigg, Lancaster, UK.

This month's fourteen new laboratory products include what may be the world's smallest glass pH electrode.

● Polyscan from Reichert Jung is a fully automatic image analysis system with interactive capabilities. The system is capable of analysing images from microscopes, video tape recorders, drum scanners, photometers and photographs. Polyscan uses a high resolution, low geometric distortion camera, capable of better than 1,000 lines, and computer controlled beam blanking for image integration. Psuedocolour processing is supplied as standard and real colour is available as an option. All set-up and operating procedures are under software control. Two cathode ray tubes are included in this system: a 12-inch black and white VDU for displaying user dialogue and programming and a 13-inch high-resolution colour monitor for the image being analysed.

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● The Plasmascan 710 spectrometer from Techmation is a fully integrated system developed for the rapid sequential analysis of most elements using atomic emission from an inductively coupled plasma source. The optical system uses two orders of the spectrum to cover the wavelength range 170 to 820 nm. Advanced direct drive technology permits 0.0005 nm per step wavelength selectability and resetability, matching the high resolution of the vacuum monochromator. Incorporated is the latest GMK nebulizer enabling analysis of high salt content solutions and geological samples without the necessity of frequent cleaning. Particles up to 1mm in diameter can be handled with ease. The spectrometer is fully controlled by the Labtam Series 3000 laboratory and general purpose computer.

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● The Model 4604 Quad 125 MHz scaler/timer/ratemeter provides high performance and flexibility for test and measurement applications as well as experimental or industrial monitoring. High 125-MHz bandwidth means superior double pulse resolution — important for low intensity industrial and control applications and vital for high intensity nuclear and particle physics experiments. Other features such as large LCD display, choice of remote control or front panel pushbutton operation and simple interfacing via RS232 to a personal computer or printer make this unit suitable for a wide variety of applications — from temporary test experiments to permanent monitors. For automatic scale normalization, the LeCroy Model 4604 may use either a preset counter or time interval. The unit also functions as a ratemeter.

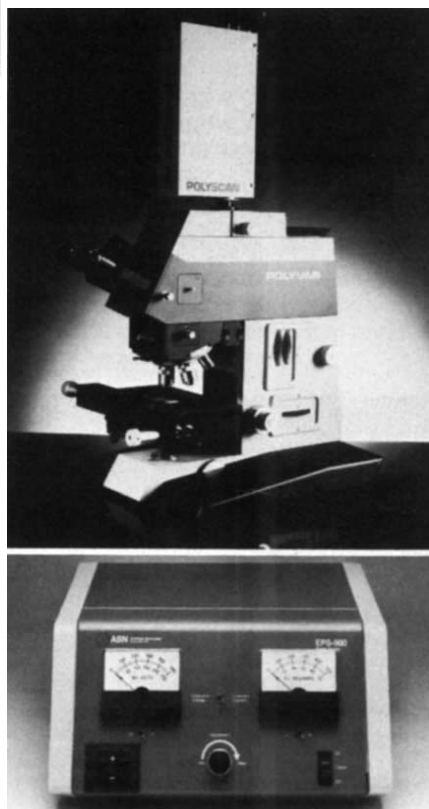
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● A durable, general purpose power supply for most routine laboratory applications, the American Bionuclear EPS-500 can provide constant voltage to 500 volts and constant current to 500mA. Standard features include output jacks to run two gels simultaneously, two dual-range meters to monitor voltage and current output, and automatic shut-off capability in the event of disconnection or user contact with the electrophoresis cell. Ideal for SDS acrylamide, agarose gels, and electrophoretic transfer techniques. Models for input voltages of both 100 and 220 V are available.

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● Kratos' new Spectroflow 783 absorbance detector combines high sensitivity performance with microprocessor-controlled automation. The instrument features variable wavelength from 190 to 700nm, and 0.001 AU full scale sensitivity with under 2% total noise. The instrument is compact, and features an attractive, spill-proof membrane front panel. Microprocessor control permits programmable automation of wavelength, sensitivity range, event mark, auto zero, and other instrument parameters.

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Reichert Jung image analysis system (top) and power supply unit from American Bionuclear.



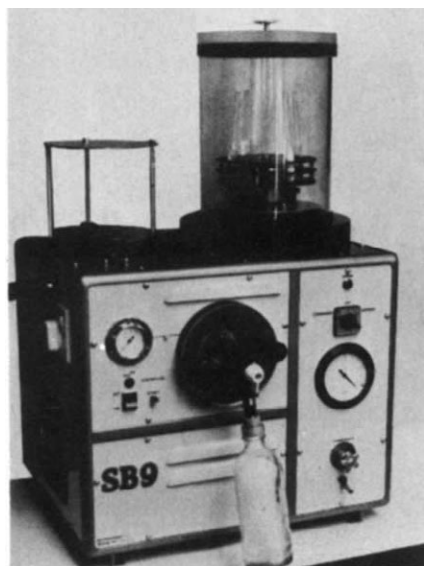
Compact power supply unit from Hoefer

● The Hoefer PS500X is a compact power supply suitable for most standard electrophoretic procedures. It operates in either the constant voltage or constant current mode, with an output range of 0 to 500 V and 0 to 400 mA. In either mode it can deliver full voltage and full current simultaneously (200 W) provided outside resistance is not limited. It may be set to cross over automatically from constant current to constant voltage when a preset voltage is reached during a run, or conversely from constant voltage to constant current. Two sets of jacks permit two experiments to be run in parallel at the same voltage. The PS500X weighs only 8 pounds and requires minimal space. It is 4 inches wide, 10½ inches deep, and 12 inches high. Separate digital meters indicate voltage and current.

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● A single-basket glassware washer capable of handling the glassware cleaning needs of 250-bed-capacity hospitals has been announced by the National Appliance Company (NAPCO). Designated Model NLW-100, the unit features three separate plumbing systems for the pre-wash, detergent wash and de-ionized rinse cycles, minimizing cross-contamination. A moving jet-spray system provides high-pressure, pulsed spraying from top to bottom for thorough penetration of even the smallest pipette. Solid-state, push-button microprocessor controls make it easy to program cycle length. The NLW-100 is constructed of corrosion-resistant stainless steel for long life and low contamination; its compact size saves on laboratory space.

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The Javac bench-top centrifugal freeze dryer

● The Javac SB9 bench-top centrifugal freeze-dryer has a 1-litre capacity condenser with a built-in spin-freeze facility. It is suitable for the drying and preservation of bacterial cultures, virus suspensions and organs such as heart valves and bone. A plastic window over the condenser allows users to observe the ice build-up on the low temperature condenser and is of particular value in the teaching of the freeze-drying technique. The standard carrier plates hold up to 116, 0.5 ml ampoules or 24, 2.5 ml ampoules. Tray drying is provided from a demountable shelf.

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● A useful guide to the hazards associated with over 500 commonly used toxic and hazardous chemicals is available from the Lab Safety Supply Co. of Janesville, Wisconsin. A glance at the chart tells you if a chemical is toxic, an irritant, corrosive, carcinogenic, flammable, explosive and much more. Also included on the chart is a synonym guide that aids in referencing common chemical names to those listed on the chart. The Chemical Hazards Chart (order no. NR CC20-2036) costs \$12.95 and is available from Lab Safety Supply Co., PO Box 1368, Janesville, Wisconsin 53547.

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● A recent addition to the Bio-Rad range is homogeneous fully active bovine brain calmodulin. The calmodulin is supplied lyophilized, salt free, in the calcium saturated form. Purity is determined by SDS-PAGE and UV analysis. Biological activity is determined by the activation of bovine heart phosphodiesterase. The new product is additional to calmodulin immobilized on crosslinked agarose bead gels, still available from Bio-Rad.

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● Calmodulin-Sepharose 4B is now available from Pharmacia Fine Chemicals. Calmodulin-Sepharose 4B is supplied as a 10 ml pack of swollen gel, with about 1 mg calmodulin per ml of swollen gel. Each batch of calmodulin-Sepharose 4B is biologically function tested with phosphodiesterase. A pure preparation of calmodulin is also available. The protein is pure by amino acid analysis, UV spectroscopy and electrophoresis. It is biologically tested with phosphodiesterase and has an activity of approximately 80,000 U per mg protein.

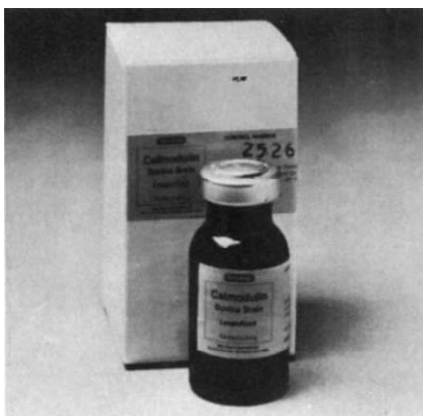
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● Prostaglandin metabolites can be measured quickly and conveniently using the new radioimmunoassay systems from Amersham International. Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ can be measured using assay systems based on 3H and ^{125}I tracers, while 'bicyclic' prostaglandin E_2 , a high stability derivative of prostaglandin E_2 , can be measured using a new 3H assay system. Each of the assay systems is completely self-contained, comprising tracer, antiserum, standards, separation system, assay buffer, protocol and results plotting sheet.

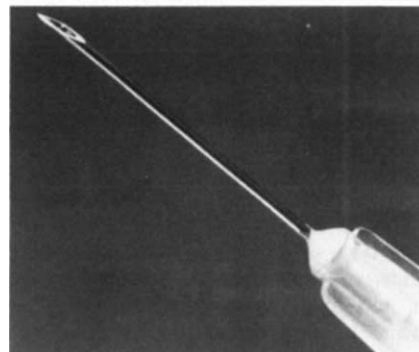
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● Version 2.2 is a new revision of Nelson Analytical's chromatography software for the IBM personal computer. The new program supports high-resolution graphics capability which more than doubles the previous graphics and screen resolution. Using the IBM monochrome display and a Hercules graphics card system screen resolution of 20×348 pixels is achieved.

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Bovine brain calmodulin added to Bio-Rad's range.



The new Anatol water analyser (top) and the advanced glass pH electrode from WPI.

● It is now possible to measure the organics content of water quickly and simply using a new analyser developed by Anatol Corporation of Colorado, USA. The A-100 analyser measures total organic carbon (TOC) via a precision electrochemical analysis method based on intense UV irradiation which breaks down the organic compounds in water. The freed carbon dioxide goes into solution as carbonic acid changing the sample conductivity which is then measured and temperature corrected to provide accurate readings down to a few parts per 10^9 . In capturing and processing TOC analysis data, the unit's sensor head provides as many as 600 data points every second which are passed to the preprocessor for calculation of average readings, screening out 'noise' in the process. Background effects are dynamically monitored and offset in final calculations. Designed for measurement of purified waters including ultrapure supplies, the A-100 features low sensitivity (10 p.p.b. detection limit, 1 p.p.b. resolution) for early warning to users and producers of water supplies.

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● World Precision Instruments, Inc. (WPI) of New Haven now claims to produce the world's smallest glass pH electrode. This electrode, mounted in the lumen of a 25 gauge hypodermic is well suited for intramuscular or intravascular insertion and prototypes have been successfully used in *in vivo* cardiac muscle experiments.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.